DREDGED MATERIAL RESEARCH PROGRAM

TECHNICAL REPORT D-77-34

AVAILABILITY OF SEDIMENT-ADSORBED SELECTED PESTICIDES TO BENTHOS WITH PARTICULAR EMPHASIS ON DEPOSIT-FEEDING INFAUNA

by

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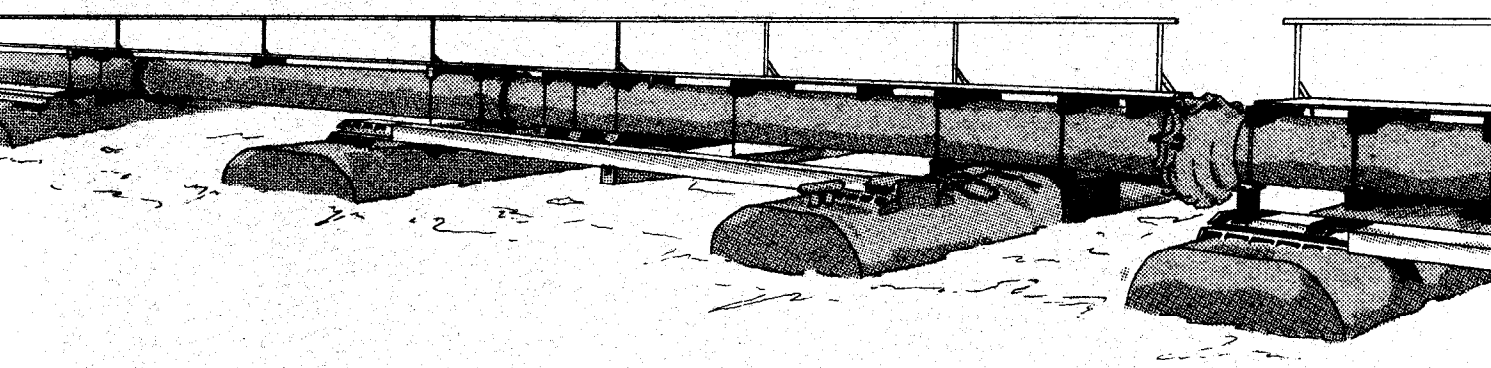

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1. The Dredged Material Research Program (DMRP) has been a broad, multifaceted investigation of the environmental impacts of dredged material disposal and has included consideration of the development of new or improved disposal alternatives. In the early stages of the DMRP's problem definition and assessment and research program development phases, it became apparent that an understanding of the actual pollution potential of dredging and discharging of sediments required substantial state-of-the-art improvement in a number of fundamental aspects. Among these was the availability of sediment-adsorbed pesticides to benthic organisms, particularly deposit feeders. Such knowledge would be useful in evaluating the potential environmental impact of disposal of pesticide-contaminated dredged material and in choosing the most desirable disposal alternative.

2. The report transmitted herewith represents the results of a research effort completed as part of Task 1D (Effects of Dredging and Disposal on Aquatic Organisms) of the DMRP. Task 1D has been part of the Environmental Impacts and Criteria Development Project of the DMRP. Among other considerations this project includes determining on a regional basis the short- and long-term effects on aquatic organisms due to dredging and discharging bottom sediment containing contaminants.

3. The objective of the reported study was to determine the ability of selective and nonselective deposit feeders to take up DDT and its degradation products DDD and DDE from sediment interstitial water and from ingested detritus or clay particles. Another objective was to determine if excretion of the pesticide and/or its degradation products would keep the levels in the organisms low or if pesticide levels in the organisms would increase gradually.

4. Sediments were artificially compounded from sand, clay, and detritus (organic matter), with clay and detritus separately tagged with radioactively labeled pesticide. Coastal and freshwater species representative of organisms common to the United States were introduced into these sediments and sampled for analysis in accordance with a predetermined time schedule.

5. Test results indicate that at least some DDT adsorbed on clay and on organic matter was biologically available, but most DDT accumulated in the tissues originated from the artificially contaminated clay. Total levels of DDT and metabolites reached a steady state after about 30 days in some species and after about 70 to 80 days in others, indicating that some type of control of internal concentration occurs. The bioaccumulation factors found for uptake from the sediments were much lower than those found where uptake is directly from water. There was also some indication that the bioaccumulation factors are not sensitive to changes in the DDT concentration of the sediment.

LIMITATIONS

6. Results of this study demonstrated that a small fraction of radio-labeled DDT freshly adsorbed to artificial sediments was available for uptake by deposit-feeding annelids. However, DMRP personnel feel that several factors should be kept in mind by those desiring to use the findings of this study to estimate potential effects in the field.

7. First among these is the general caution that no laboratory study can exactly duplicate field conditions; therefore, only trends, rather than precise response, can be extrapolated to the field. The entire study was conducted with artificially prepared sediments labeled with radioisotopes rather than with contaminated natural sediments. An artificial organic substrate was used as substitute detritus with no attempt to verify its suitability. There was no measurement of initial DDT body burdens in the test animals collected, although this could affect regulation or uptake processes, nor was there any measurement of total body burden after the experimental exposure period. The observed body-burden levels were combinations of DDT actually incorporated in the body tissue and DDT passively being transported through the gut still tightly adsorbed to sediment particles. Therefore, results from the so-called "tissue analyses" are artificially high by an unknown amount and the actual extent of DDT accumulation in organisms tissue was not determined.

8. The study demonstrated that a viable pathway exists for the movement of radio-labeled DDT from freshly tagged artificial sediments to benthic organisms. However, in view of the above comments, the reader is urged to consider carefully the appropriateness of this study to his needs before the results are used to assess the significance of such movement.

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Colonel, Corps of Engineers
Commander and Director

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As part of the Dredged Material Research Program, Task ID: Effects of Dredging and Disposal on Aquatic Organisms, a study was undertaken to determine the availability of sediment adsorbed DDT (and its metabolites) by several species of deposit-feeding benthic infauna that may form a link for the entry of DDT into aquatic food webs. The experimental species studied were Capitella capitata, a nonselective feeding marine polychaete; Nephtys californiensis, a selective feeding marine polychaete; and Tubifex tubifex, a nonselective feeding freshwater oligochaete. Sediments were artificially composited (Continued)

20. ABSTRACT. (Continued)

from silica sand, clay, and aged baby cereal for Tubifex, and from fired beach sand, clay, and aged baby cereal for Capitella. The clay and cereal components were tagged separately with radioactively labeled DDT prior to mixing with the sand, the final theoretical concentrations being 1 ppb ^{14}C-labeled DDT and 1 ppb ^{3}H-labeled DDT. For Nephtys the sediment of its natural habitat was tagged with ^{14}C-labeled DDT to a final concentration of 0.6 ppb. Tagged sediment was placed in stacking dishes, to which the test organisms were added. The stacking dishes with the test organisms were placed in aquaria with constantly flowing water. The experiments were conducted in two replications with controls. Sampling consisted of removing stacking dishes from the aquaria at predetermined times removing the organisms from the sediment and placing the organisms in water for about an hour allowing them to at least partially void their gut. Samples were then inserted into vials and frozen until analysis. Extraction of DDT and its metabolites was done in accordance with standard EPA procedures. Separation of DDT and metabolites was effected by thin-layer chromatography, while quantification was done by liquid scintillation counting of the ^{3}H and ^{14}C.

DDT with both labels was found to accumulate in Capitella and in Tubifex. This indicates that at least some of the DDT is available when adsorbed on clay and on organic matter. Accumulation was also found in Nephtys, but most of the DDT originated from clay as suggested by the low (0.06%) organic carbon content of the sediment and by the results of a separate experiment in which clay only was tagged.

Combined uptake of DDT and metabolites reached a steady state in Capitella and Tubifex after about 30 days and in Nephtys after about 70 to 80 days, indicating that some type of control of internal concentration occurs. The bioaccumulation factors found were about 2 for Tubifex, about 50 to 70 for Capitella and about 8 for Nephtys. These factors are much lower than those found where uptake is directly from water. There is also some indication from Tubifex experiments that the bioaccumulation factors are not sensitive to changes in the DDT concentration of the sediment.

The degradation of DDT, to the extent that it occurred, was almost entirely to DDD. Only in Tubifex was there some evidence of degradation to DDE. The rate of degradation in the sediment appeared to be first order with respect to DDT, but too few data were taken to determine the rate constant accurately. The DDT/DDD ratios in the organisms were about the same as in the sediment in the samples analyzed.

Additional experiments are suggested to generalize the bioaccumulation factor concept for the uptake of DDT and pesticides in general and to clarify further the kinetics and mechanism of uptake.

Additional experiments are suggested to provide more quantitative data regarding the uptake of DDT and other pesticides by deposit feeders in contaminated natural sediments.

DISCLAIMER

The primary objective of Contract DACW39-74-C-0103 was to determine the availability of sediment-associated chlorinated hydrocarbon pesticides to deposit-feeding infauna. This was to include determination of uptake from interstitial water, mineral particulates, and organic detritus, as well as an estimate of the degree of bioaccumulation. Results of the study demonstrated that a small fraction of freshly added radiolabeled pesticide was available for uptake by benthic organisms.

The Dredged Material Research Program personnel feel several factors should be kept in mind by those desiring to use the findings of this study to estimate potential effects in the field. First among these is the general caution that no laboratory study can exactly duplicate field conditions and therefore the best-designed laboratory studies usually permit only the extrapolation of trends, rather than precise response, to the field. Several specific points concerning this study should also be noted. The entire study was conducted with artificially prepared isotopically labeled sediments rather than with contaminated natural sediments. An artificial organic substrate was used as substitute detritus with no attempt to verify its suitability. This difficulty is acknowledged on page 38 of the report: "Whether or not one can draw the conclusion that DDT is a little more available from organic matter than from inorganic matter is problematic because of the artificiality of the organic component of our sediment."

Several points concerning pesticide analyses should also be considered. There was no measurement of initial DDT body burdens in the test animals collected, although this could affect regulation or uptake processes, nor was there any measurement of total body burden after the experimental exposure period. Also, after noting an apparent substantial loss of DDT from the sediments, the authors (page 40) "...believe most or all of this apparent decrease not to be real, but to be the result of an extraction efficiency lower than indicated by our blank experiments...." They dismissed the possible loss of DDT to the water column even though the concentration that would be required

to produce the observed apparent loss in the flow-through system used would only be less than 0.003 ng of DDT per liter as shown by the following computation, which is based on data from Table B8:

$$\frac{(400 \text{ g sediment})(0.6 \text{ ng DDT/g} - 0.24 \text{ ng DDT/g})}{(432 \ell/\text{day})(120 \text{ days})} = <0.003 \text{ ng DDT}/\ell$$

Since this loss is less than the detection limit in the study, it may be that loss to the water column was too readily dismissed. This point is important because it will affect the calculated accumulation ratios and calculated degradation rates and may even influence the observed DDT uptake since material in the water column is generally more available than the same material in a particulate phase.

The reader is also cautioned that the observed body burden levels are a combination of DDT actually incorporated in the body tissue and DDT passively being transported through the gut. Prior to analyses, the test organisms were purged in clean water for only a short period and, according to page 48, "...complete voiding of the guts probably did not occur...." Therefore, results from the so-called "tissue" analyses are artifically high by an unknown amount and the actual extent of DDT accumulation in organism tissue was not determined.

The data did not receive adequate statistical treatment and most discussions should be considered qualitative rather than quantitative in nature. For example, the data on tissue plus gut concentration versus exposure time in Figures 6-8 should have been subjected to regression analysis and then compared to model predictions. Instead, the predictions plotted with the data were constructed from a model derived from the same data that the curves appear to summarize.

The study demonstrated that a viable pathway exists for the movement of radiolabeled DDT from freshly tagged artificial sediments to benthic organisms. However, in view of the above comments, the reader is urged to consider carefully the appropriateness of this study to his needs before the results are used to assess the significance of such movement.

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PREFACE

The work described in this report was performed under Contract No. DACW39-74-C-0103, titled "Study to Determine the Availability of Sediment Adsorbed Selected Pesticides to Benthos with Particular Emphasis on Deposit Feeding Infauna," dated March 29, 1974, between the U. S. Army Engineer Waterways Experiment Station (WES), Environmental Effects Laboratory (EEL), Vicksburg, Mississippi, and LFE Environmental Analysis Laboratories Division of LFE Corporation. The study was sponsored by the Office, Chief of Engineers (DAEN-CWO-M) under the civil works research program, "Dredged Material Research Program."

This report describes the results of studies of DDT uptake and its metabolites by three diverse species of annelids from artificial and natural sediments tagged with radioactively labeled DDT, discusses the data in terms of a dynamic uptake model and bioaccumulation factors, and presents some factors which could be considered for the establishment of dredged material disposal criteria.

The study was conducted under the project leadership of Dr. M. W. Nathans of LFE Environmental in Richmond, California, assisted by Dr. T. J. Bechtel of CH_2M-Hill in Bellevue, Washington. Significant contributions by Messrs. H. Y. Gee, senior chemist, and J. C. Corso and K. Leung, laboratory technicians of LFE Environmental, by Messrs. R. H. Johnston, D. Wisegarver, and H. Johnson, biologists, of CH_2M-Hill, and by Dr. K. Tenore, Woods Hole Oceanographic Institute, Dr. A. W. Carey, Oregon State University, and Mr. L. Birke, Northwest Pulp and Paper Institute (formerly of CH_2M-Hill), consultants, are acknowledged.

The contract was monitored by Ms. Pat Kerr, Ecosystem Research and Simulation Division, EEL, and Ms. Susan Palmer and Dr. R. H. Plumb, Jr., Environmental Impacts and Criteria Development Project, EEL. The study was under the direct supervision of Dr. R. M. Engler, Project Manager, Environmental Impacts and Criteria Development Project, and the general supervision of Dr. John Harrison, Chief, EEL.

Contracting Officers were Col. G. H. Hilt, CE, and Col. J. L. Cannon, CE. Technical Director was Mr. F. R. Brown.

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CONVERSION FACTORS, U. S. CUSTOMARY TO METRIC (SI) UNITS OF MEASUREMENT

U. S. customary units of measurement used in this report can be converted to metric (SI) units as follows:

Multiply	By	To Obtain
Pounds	453.6	Grams
Gallons (U. S. liquid)	3.785412	Cubic Decimeters

AVAILABILITY OF SEDIMENT-ADSORBED SELECTED PESTICIDES TO BENTHOS WITH PARTICULAR EMPHASIS ON DEPOSIT-FEEDING INFAUNA

I. INTRODUCTION

One of the major responsibilities of the U. S. Army Corps of Engineers is to keep waterways and harbors navigable by dredging. Subsequent disposal of the dredged material has become a potentially significant environmental problem that the Corps must address more thoroughly. Disposal areas within reasonable distances of dredging activities are becoming filled or are being reevaluated as to how the land ultimately should be utilized. In addition, the more stringent regulations recently promulgated concerning dredged material disposal (i.e., Public Law 92-500, Federal Water Pollution Control Act Amendments of 1972, and Public Law 92-532, Marine Protection, Research, and Sanctuaries Act of 1972) have led to development of preliminary dredged material disposal criteria (Keeley and Engler, 1974). However, a data base is currently lacking upon which meaningful final criteria can be developed for evaluating the relationship between dredging activities and environmental impact (Lee and Plumb, 1974).

Initial criteria were expressed as the maximum concentration of certain parameters that would be allowed in dredged material. No distinction was made, however, between the fraction of each toxicant that is available to aquatic organisms and the fraction that is not. It is important to make this distinction as only the available fraction may have an impact on flora and fauna at a disposal site. For example, significant amounts of persistent pesticides can be found in estuarine and riverine sediments that must be periodically dredged. It is possible that deposit feeding organisms in a dredged material disposal area could accumulate pesticides from the contaminated material after colonization if the pesticide or a fraction thereof is available. Potential effects of accumulation include acute and chronic toxicity to the organism and biomagnification of the pesticide residue in aquatic food webs through predation and recycling.

This study is one of a series of research studies being conducted by the Dredged Material Research Program (DMRP) of the U. S. Army Corps

of Engineers Waterways Experiment Station (WES). The overall objective of the DMRP is to provide more definitive information on the environmental aspects of dredging and dredged material disposal operations and to develop technically satisfactory, environmentally compatible, and economically feasible dredging and disposal alternatives (Office of Dredged Material Research, 1974).

The original objective of this study was to determine the ability of selective and nonselective deposit feeders to take up DDT and its degradation products (DDD and DDE), chlordane and malathion. Included in this objective was the determination of the availability of these pesticides from interstitial water and from ingested detritus (for selective feeders) or from detritus plus clay particles (for nonselective feeders). Another objective was to determine if uptake and accumulation, if it occurs, could be controlled by internal regulation, that is, if excretion of the pesticides and (or) their degradation products would keep the levels in the organisms low, or if pesticide levels in the organisms would increase gradually as a result of lack of internal regulation.

It was intended to achieve the objectives by a three-phase program. In the first phase sediments were to be artificially compounded from sand, clay, and detritus (organic matter), with clay and detritus to be separately tagged with radioactively labeled pesticide. Coastal and freshwater species were selected that were common to the United States. Organisms were to be introduced into these sediments and sampled for analysis in accordance with a predetermined time schedule. The results of these Phase I experiments would indicate the ability of the organisms to take up pesticides from the components of the sediments, and would also indicate whether or not regulation would occur.

The translation of the results to naturally occurring situations was to be accomplished in two steps. In Phase II the experiments were to be repeated with artificially tagged natural sediments, and in Phase III organisms and sediment samples were to be gathered in a predetermined time sequence from contaminated sediments and analyzed.

During the execution of the first phase, which was novel with regard to the way in which radioactive tracer techniques were applied to the compounding of sediments and to the particular species selected, it became

apparent that the entire program could not be completed within the bounds set by the Waterways Experiment Station. Furthermore, some preliminary experiments indicated that satisfactory tagging of sediments with chlordane and malathion was not possible. Whereas DDT was virtually quantitatively adsorbed by the clay and the organic components of the sediments, chlordane and malathion were not as adsorbed. Thus the availability from organic and inorganic components and from interstitial water separately could not be investigated. Therefore, we are reporting here only on the methodology and the results of laboratory experiments with organism in sediments artificially tagged with DDT. Additional studies should be conducted with tagged uncontaminated natural sediments and with contaminated sediments in order to allow translation of our results to naturally occurring situations.

II. METHODS AND MATERIALS

A. Study Area

A mobile trailer laboratory was used to conduct the uptake experiments in Westport, Washington. A site on the south jetty bordering the entrance to Grays Harbor was selected for the laboratory to provide access to high quality seawater. In addition, this site was located close to broad, sandy beaches between Grays Harbor and Willapa Bay to the south, as well as to the extensive mudflats within Grays Harbor, both of which provided suitable habitats for the benthic species to be utilized in the study.

B. Mobile Laboratory

The laboratory was a 13.7 x 3.6 m mobile trailer designed for routine water chemical analyses as well as continuous flow and static bioassays. Both seawater and municipal fresh water could be simultaneously passed through separate continuous-flow systems (Figure 1).

The seawater continuous-flow system consisted of a 2.5-cm-diameter polyvinylchloride (PVC) pipe extending into Grays Harbor on the east side of jetty No. 6, a 1.5-horsepower intake pump (Sears Hydrojet), a 1.9-m^3 stainless steel tank, and an electric float switch. The intake was located 15 m from shore in 4.5 m of water at mean lower low water.

The intake pump (capacity 227 liters per minute) was located on

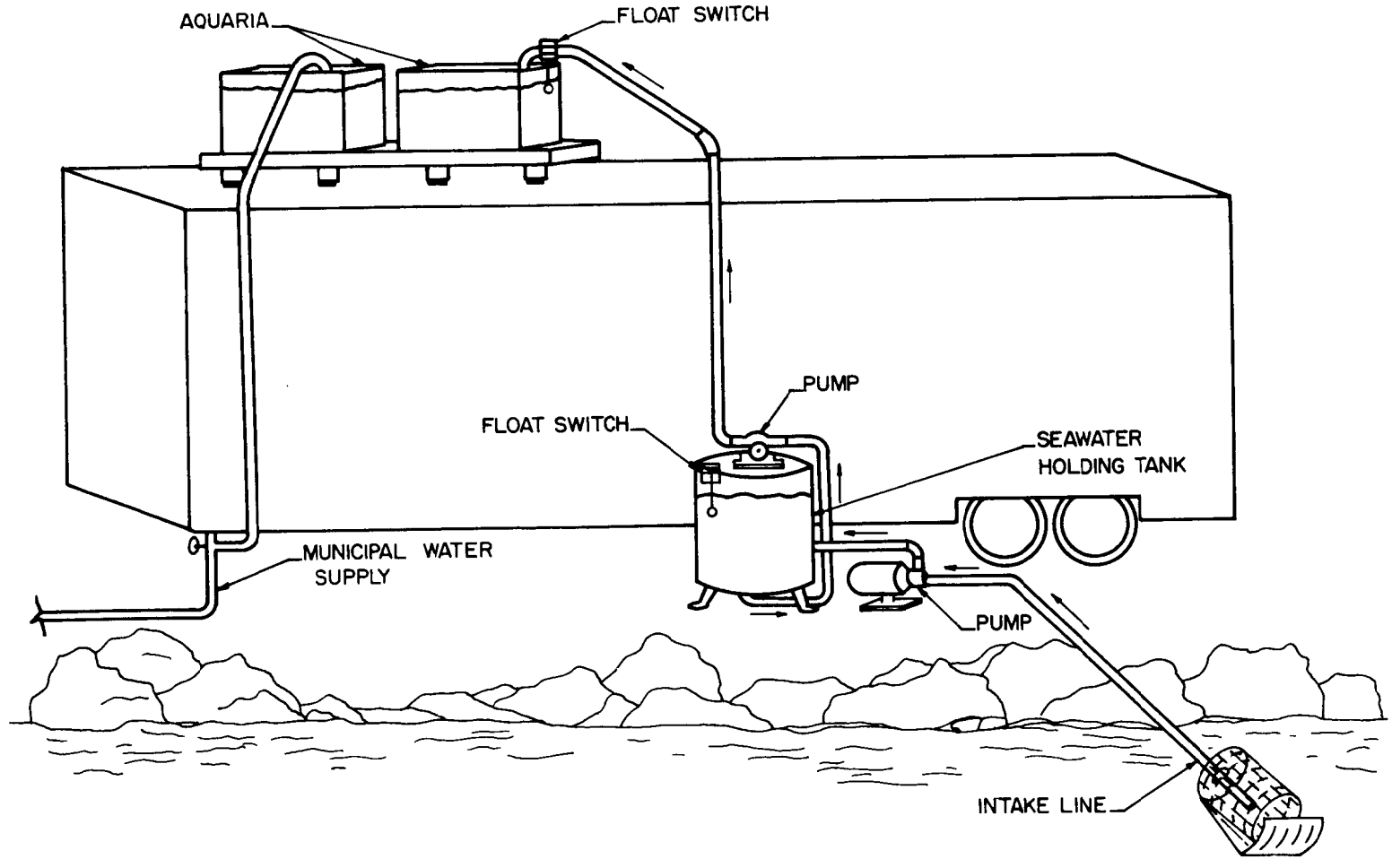

Figure 1 Mobile research laboratory continuous-flow supply system

shore between the supply line and the stainless steel tank, which was utilized as a reservoir in case of an intake-pump failure. The electric float switch situated within the stainless steel tank was used to activate the intake pump when the reservoir volume fell below 1.5 m^3. From the holding tank, water was pumped with a Sears Handy Pump(R) through a PVC pipe having a 1.9-cm diameter to a 208-liter glass aquarium, located on the roof of the trailer in a wooden box insulated with styrofoam. The pump was operated by a float switch in the aquarium.

The municipal freshwater supply was received directly from the City-of-Westport system via a garden hose connected to an intake line underneath the trailer. Water pressure was great enough to force the water to a second 208-liter glass aquarium, which served as a holding tank on the roof of the trailer.

From each of the aquaria, water flowed by gravity into the delivery system inside the laboratory (Figure 2). The delivery system utilized a constant head mechanism to maximize constant flow rates. This mechanism consisted of a head box connected to a PVC pipe 5.1 cm in diameter. This pipe was fitted with rotatable glass tubes, which emptied directly into funnels located above 75.7-liter aquaria. A constant water volume in the head boxes was maintained by adjusting PVC valves in the delivery lines. Flow rates to individual aquaria were controlled by adjusting the angle of rotation of each glass tube.

For experiments in which fresh water from the Johns River, a stream draining forested lands to the south of Grays Harbor, was used, a recirculating system was constructed to sustain continuous flows (Figure 3). It was not possible to have once-through flow due to the logistics of transporting approximately 570-liter of river water to the laboratory each day. By making one daily water collection trip, about 33% of the volume of the entire system was replaced each day.

Each aquarium used as a test chamber during the uptake studies contained a standpipe to maintain a constant water level within the tank. Standpipes of various lengths were tested to determine the flow rate that would provide adequate water circulation to maintain the test organisms. Standpipes 7 cm high were eventually selected to create constant volumes

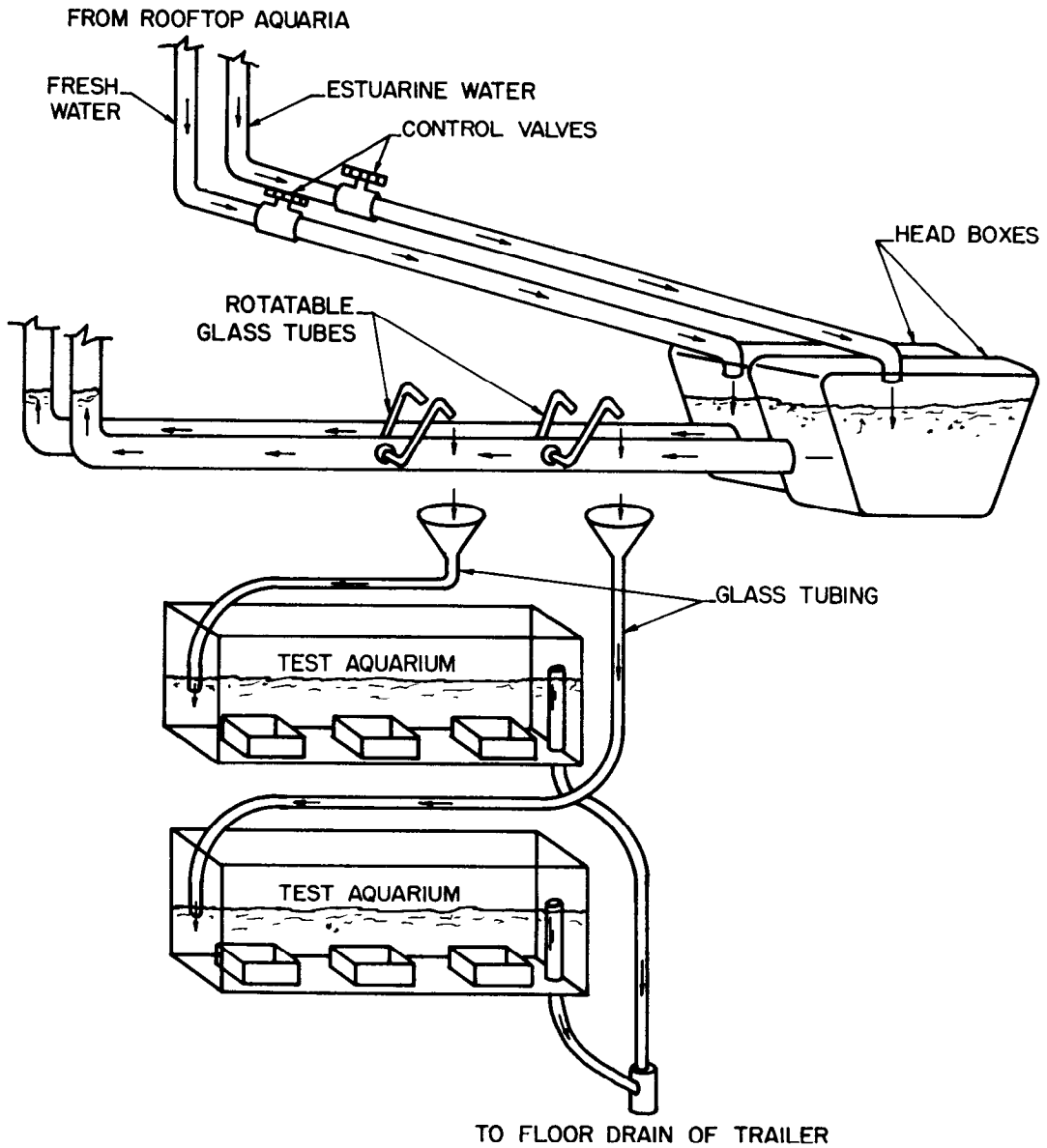

Figure 2 Mobile research laboratory continuous-flow delivery system

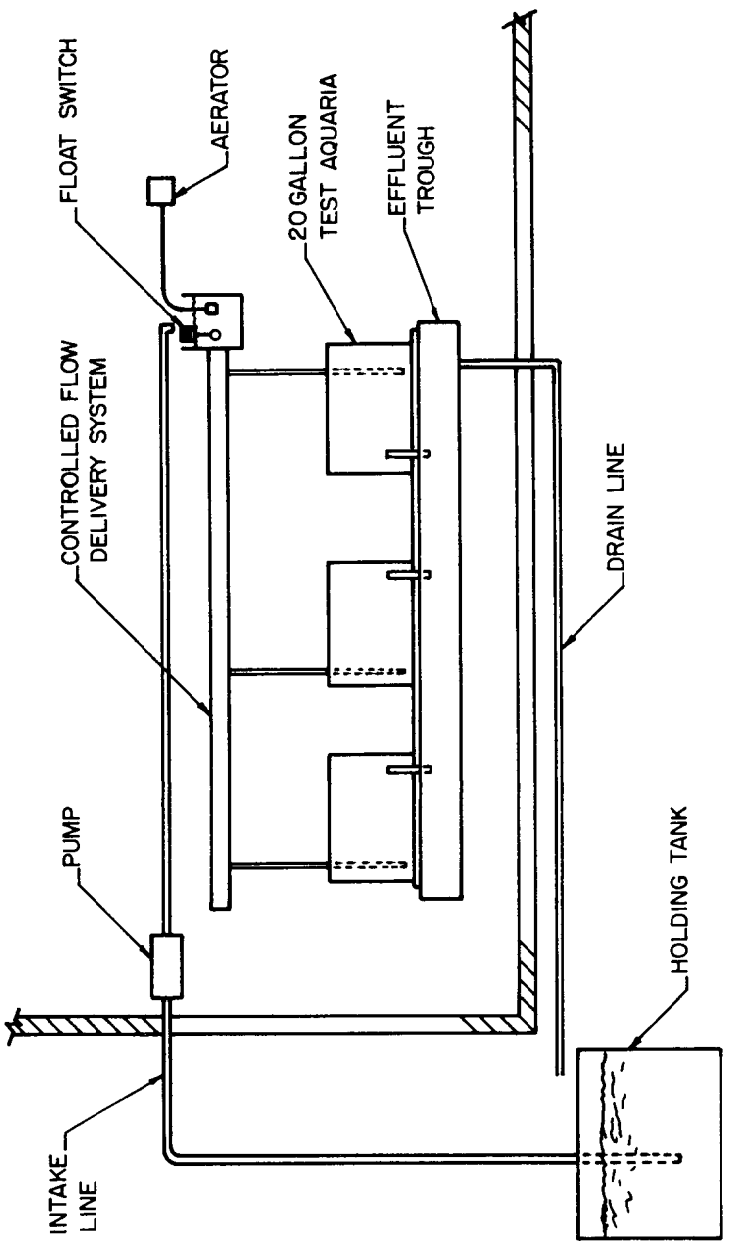

Figure 3 Freshwater recirculating system

of 15.7 liters in the aquaria. As flow rates were maintained at 0.3 liters per minute, the exchange rate of water in the aquaria was 27.5 volumes per day.

C. Field Collections

The organisms selected for this study represented selective and nonselective benthic deposit-feeding infauna from marine and freshwater environments. The marine polychaete Nephtys californiensis commonly found in the study area was assumed to represent a selective feeder. Nephtys was collected in the intertidal zone on sand-tidal flats in Willapa Bay and Grays Harbor. After shovels were used to overturn the sand, the sand piles were separated by hand. Any worms found were quickly placed in buckets containing approximately 10 cm of the worm's natural substrate and 15 cm of seawater. Upon return to the laboratory, the worms were placed in holding aquaria connected to the continuous-flow seawater system to allow them to acclimate in approximately 10 cm of the natural substrate.

The nonselective marine feeder used in the study was the polychaete Capitella capitata. This species was found to be abundant in the soft mud of Westhaven Cove, adjacent to the laboratory. Sediment samples were collected from various boat docks in the cove with an Ekman dredge and placed in buckets. Upon return to the laboratory, worms were removed from the mud by hand and placed in glass dishes containing 2-4 cm of natural beach sand, which had been baked at 400° C for several hours. The dishes were then placed in aquaria connected to the continuous-flow seawater system.

The freshwater species used in the study was the common, nonselective feeding sewage worm, Tubifex tubifex. They were purchased from the Fish Factory (Seattle, Washington), transported to Westport, and placed in glass dishes containing 2.5 to 3 cm of an artificial substrate. The dishes were then placed in aquaria connected to the continuous-flow municipal freshwater system. The aquaria were aerated by means of airstones to remove chlorine. The artificial substrate consisted of 85% by volume natural grain silica sand (Ottawa Silica Company, Ottawa, Illinois), 10% pulverized clay (Edgar Plastic Kaolin Co., Edgar, Florida), and

5% Pablum (Gerber Mixed Cereal for Baby, Gerber Products Co.), which served as a source of organic material.

Tubifex and the two marine species were acclimated in the aquaria for at least three days before transfer to experimental aquaria.

D. Experimental Design

The original intent was to use artificial sediments compounded from silica sand, clay, and aged cereal, with the clay and the cereal separately tagged with pesticide. Cereal was selected as a suitable substitute detritus for benthic worms based on procedures used by Dr. Kenneth R. Tenore, Woods Hole Oceanographic Institute (1974), who cultured Capitella in mixtures of baby cereal and sand before utilizing them in flounder food chain studies. Initially the composition of sediments was, by weight, 98% silica sand, 1% clay, and 1% cereal. It was observed, however, that Nephtys was not feeding well in this artificial mixture. Hence, after some experimentation, including confirmation of Nephtys being a selective feeder by microscopic examination of its gut content, the decision was made to tag the natural sediment inhabited by this species, as the cereal appeared to be an unsuitable food source. The tagging methodology is discussed in the sediment preparation section.

It was further found that the amount of cereal used created toxic conditions for Capitella capitata. Several tests were conducted substituting the mud from which Capitella was collected and sterilized natural beach sand for silica sand, because the properties creating the toxicity could not be identified. In addition, the amount of aged cereal was reduced and the pesticide concentration on the cereal increased. Problems with Capitella survival were eventually solved and an artificial sediment consisting of cereal, clay, and sterile beach sand was selected for the long-term experiment. There was no evidence that Capitella was adversely affected after the amount of cereal was reduced.

In contrast to the problems encountered with Nephtys and with Capitella, no problems were encountered with Tubifex tubifex.

Tritium labeled DDT in a benzene solution was obtained from New England Nuclear Corp. in benzene solution. Its specific activity was 44.4 m Ci/mmole (8 mg/mCi). DDT labeled with ^{14}C, also in benzene

solution, was purchased from Amersham. Its specific activity was 32.3 mCi/mmole (11 mg/mCi).

To ensure the least possible bias from sampling error and to prevent disturbance of the population during sampling, sediments and worms were placed in individual stacking dishes approximately 11.4 cm in diameter by 4.4 cm deep. Each dish constituted a single sample. Two Nephtys, 15-20 Capitella, and about 150 Tubifex were placed in each dish to provide an adequate amount of biomass for analysis. One aquarium contained control dishes, and two aquaria were used for the tagged sediments. The control tanks contained eight dishes while each of the replicates held up to fourteen. Generally more dishes were placed in the tanks than the number of samples to be collected in order to compensate for mortality, escape from the dish (Nephtys only), or an extension of the sampling schedule.

E. Sediment Preparation

To achieve uniform pesticide distribution in the dishes, sediments in each dish were tagged and subsequently mixed individually. The tagging procedures do not guarantee that the DDT is incorporated in the sediments in the same manner as in "naturally" occurring polluted sediments, although adsorption is the only way by which clay can contain DDT. The existence or absence of any such equivalence between the artificial sediments and natural sediments is very difficult, if not impossible, to prove. This is one of the reasons that additional experiments should be performed to translate our results to naturally occurring situations.

Artificial sediments for Tubifex experiments and preliminary experiments with Capitella and Nephtys were created by weighing clay aliquots equivalent to 1% of the total 400 g of sediment (on a dry weight basis) into stacking dishes and adding 2.5 ml of water (fresh for Tubifex and seawater for Nephtys and Capitella). Three ml of water per gram of clay were then added to a small beaker, followed by the addition of an appropriate amount of DDT to obtain the desired level of labeling. The contents of the beaker were added to the clay aliquot drop by drop with constant swirling. Mixing continued for 1 minute after completing the addition of the spike.

Cereal was aged in a little water for several days before use. Aliquots of this aged cereal equivalent to 1% of the sediment by weight were

weighed in beakers and 5 ml of water per gram of cereal were added to the beaker. (In some experiments the amount of cereal used amounted to 0.1% of the sediment by weight.) The cereal was then aged at least one additional day before spiking. The spiking procedure was the same as that used for clay, except that the spike was added to 5 ml of water per gram of cereal.

Aliquots of sand equivalent to 98 percent of the total sediment were weighed out and approximately one-half of the sand was mixed with the clay aliquot. Additional water was then added as needed to just dampen the mixture. All of the cereal aliquot was added and thoroughly mixed. Approximately 80% of the remaining sand was then added and mixed with just enough water to moisten the mixture. The remaining sand was then sprinkled on top to minimize disturbance of the labeled sediment when the dish was placed in the aquarium.

The total weight of sediment used in preliminary experiments was 400 g per sample for Nephtys and 200 g per sample for Capitella and Tubifex. For final experiments, the total weight of sediment used for all species was 400 g.

The basic procedure for preparation of tagged natural sediment for Nephtys experiments was the same as for the artificial sediments. Natural sediment was sieved to remove extraneous material. Aliquots equivalent to 1% by weight of the total sediment were weighed out and tagged as previously described for clay. The tagged aliquots were then completely mixed with the untagged remainder of the sediment.

The procedure for tagging sediments for Capitella experiments was the same as that used for Tubifex except that sterile beach sand was substituted for silica sand.

F. Sampling Procedures

After water was added to within 1.3 cm of the lip of the stacking dishes containing the experimental and control sediments, worms were transferred from the holding tanks to the dishes. The dishes were then randomly placed in the respective experimental and control aquaria. Preliminary data had dictated some minor changes in original sampling times. For the final long-term experiments, the sampling schedules were as follows: Tubifex, 12 hr, 1, 2, 3, 5, 9, 12, 17, 22, 27, 32, 37, 46, and 52 days; Nephtys, 12 hr, 1, 2, 4, 8, 12, 16, 24,

31, 39, 48, 58, 68, 78, 88, 103, 119, and 125 days; and Capitella, 12 hr, 1, 2, 8, 12, 18, 22, 27, 33, 37, 42, 49, and 56 days.

The behavior of the test organisms was observed and any apparent abnormal behavior noted throughout the course of an experiment.

At each sampling time, dishes containing worms were randomly removed from the experimental and control tanks. Worms were individually picked from the containers with forceps and placed in a glass beaker containing 10 ml of water. Any worms that did not appear to be normal or that were lying on the sediment surface were not collected to eliminate the possibility of analyzing dead worms. After being allowed to void their guts in the beakers for one hour, the worms were placed on Kimwipes(R) to remove excess water and then frozen in glass vials. The water and feces remaining in the beakers were transferred with disposable glass pipets to glass vials and frozen.

Sediment samples were collected with a cork borer from dishes without worms in the aquaria, placed in glass vials, and frozen. These samples were collected to determine if any changes in pesticide concentration in the sediments were occurring independent of any influence of burrowing worms. The sediments in the dishes in which the worms had been burrowed were scraped into glass jars with a rubber policeman and frozen.

Water samples were collected from each aquarium in 2-liter glass jars and refrigerated. After all samples had been collected, they were packed in dry ice and shipped to LFE Environmental in Richmond, California, for analysis.

G. Analytical Procedures (DDT Only)

1. Tissue Samples

Each batch of tissue samples that was received was analyzed in order, starting with the sample expected to contain the lowest concentration, i. e., the first sample collected.

The procedure was an adaptation of the standard EPA procedure (Thompson, 1974). Qualitatively, the individual steps were as follows:

a. The samples were ground in a mortar while still frozen.

b. Saturated Na_2SO_4 and an equal volume of water were added, and the mixture transferred to a centrifuge cone. The residue remaining in the mortar was washed out with a small amount of water and added to the centrifuge cone. Further washing was accomplished with a few milliliters of hexane-acetone (4:1) mixture, followed by a second water-and-solvent washing cycle.

c. The DDT (and metabolites DDD and DDE) were extracted into the organic phase by stirring with a glass stirring rod with a specially designed impeller. The organic phase was transferred to a 50-ml beaker.

d. Step (c) was repeated once with a fresh aliquot of organic solvent; then repeated with pure hexane. The yield of the extraction was about 90% as determined by comparison with a spike.

e. The organic extract was air-dried to wet-dryness, and the residue transferred to a tared vial with a little pure hexane (sp gr 0.66).*

f. The contents of the vial were allowed to evaporate to about 1 ml at room temperature and then weighed to obtain the volume.

g. An aliquot was pipetted into 10 ml "Aquasol" liquid scintillation counting cocktail purchased from New England Nuclear and counted for either 50 minutes or 100 minutes in a Tracerlab Corumatic liquid scintillation counter with discrimination between ^{3}H, ^{14}C, and ^{32}P channels.

h. Another aliquot was injected into the gas chromatograph, a Hewlett-Packard model 5700 with electron capture detector. The conditions were as follows:

Column: 4' x 1/4" glass, OV-1 on Chrom-W, 100/200 mesh

Column Temperature: 200° C

Injector Temperature: 250° C

Detector Temperature: 350° C

Flow: 120 ml/min (A-CH_4)

*Cleanup in a florisil column is not adequate. Later work has shown that cleanup by TLC is the preferred procedure.

Alternatively, an aliquot was placed on a TLC plate together with aliquots of DDT, DDD, and DDE standards. The plate was precoated with a 0.25-mm-thick layer of Silicagel F-254. The solvent used was n-heptane with 1% acetone. The migration period was 15 minutes, with the front traveling 41 mm. The R_f values (migration distance relative to the solvent front) were as follows:

Component	R_f found	R_f from literature*
DDE	0.68	0.65
pp - DDT	0.47	0.48
DDD(TDE)	0.32	0.32

i. Total tissue weight was obtained by weighing the original container as received and again after removal of the sample. This weight was corrected for traces of sediment adhering to the samples by separating the sediment residue from the aqueous phase left after extraction by centrifugation and washing, slurrying with ethanol, transferring the slurry to a tared crucible, air-drying, igniting to 600° C in a muffle furnace, and reweighing the crucible. In general the amount of pesticide estimated to be associated with the sediment residue was small compared to the total amount of pesticide measured.

Although the smaller clay particles and perhaps also the organic components may have adhered to the worms preferentially, it was assumed for the weight-correction procedure that the weight of the organic sediment component ashed together with the tissue residue was small compared to the total weight of the adhering residue. It is to be noted in this connection that the specific gravity of the organic component (about 1) is much smaller than that of the inorganic components of the sediments (about 2.5).

Corrections to the tissue weight resulting from residual water in the frozen samples were not made. Examination of the samples indicated that this correction would have been small, however. No adequate and valid means of determining the recovery were found.

*Kovacs (1965)

2. Sediment Samples

Sediments were air-dried in a large Petri dish and weighed. After homogenization an aliquot was taken, weighed, and transferred to a filter paper for insertion into a Soxhlet extractor. Pesticide extraction was accomplished with a hexane-acetone mixture. The solvent was removed and analyzed in the same manner as tissue samples except that a cleanup step with a florisil column was added. The recovery averaged about 85% in blank experiments.

3. Water Samples

DDT was extracted from water samples with ethyl ether (15%) - hexane, in two cycles. A third extraction cycle was performed with pure hexane and a saturated Na_2SO_4 solution. A florisil cleanup step was added when necessary. The remainder of the procedure was the same as that for tissue and sediment samples. The recovery was in excess of 80%.

4. Fecal Samples

Fecal samples were analyzed in the same manner as tissue samples, except that the intial grinding step was omitted. Gross weight and contaminant sediment weight were determined, the latter to estimate the correction to the pesticide content due to adsorbed sediment particles. The true weight of the fecal matter could not be determined.

5. Measurements

Gas Chromatography (GC) - DDT and metabolite peaks were measured and compared with peaks obtained from standard injections. Normally the sensitivity of the DDT measurement is 0.001 ng. The tissue samples exhibited a series of rather broad background peaks that reduced the sensitivity considerably. The normal cleanup procedure with the florisil column did not sufficiently remove the material causing these peaks. Furthermore, the peak heights and areas varied, being dependent, in part, on the sample size. Consequently, at low DDT and metabolite concentrations, the precision and accuracy of the GC measurements were unsatisfactory.

Liquid Scintillation Counting - The liquid scintillation counting (LSC measurements) is usually not affected by the lack of selectivity of the pesticide analysis procedure as practiced. The sensitivity is comparable to the theoretical sensitivity of gas chromatography, i.e., about 0.001 ng for the ^{14}C-labeled DDT and about 0.003 ng for the ^{3}H-labeled DDT. These sensitivities are determined by the specific activities of the labeled compounds, the counting time, and the characteristics of the counter. With regard to the counter characteristics, the following is mentioned: because of degradation of the beta energies, there is a significant overflow of ^{14}C beta energies into the ^{3}H channel of the LSC. However, the overflow of ^{3}H beta energies into the ^{14}C channel is small. Consequently, when both ^{3}H- and ^{14}C-labeled compounds are used, the counts in the tritium channel must be corrected for the presence of counts originating from the ^{14}C activity. This correction is determined by means of a graph constructed from data obtained by counting standards having count rates covering the range encountered in the experiments. No correction of the ^{14}C counts for the overflow from the ^{3}H channel is necessary, because this correction is less than the experimental variation, with the relative ^{3}H and ^{14}C count rates used. Counting efficiencies were 77% for ^{14}C and 55% for ^{3}H.

In a few cases, but only with some of the Nephtys samples, the contaminants accompanying the DDT and its metabolites caused a slight coloration of the cocktail, resulting in a decrease in the counting efficiency. The required correction is made by recounting the sample with a known activity spike added.

III. RESULTS

A. Preliminary Studies

1. System Characterization

Background levels of DDT and its metabolites were found to be at or below the detection limits (0.01 - 0.001 ng/g) by gas chromatographic analysis. Levels of PCB's were mostly at the detection limit (0.01 ng/g) but were slightly elevated (to 0.1 ng/g) in a small number of samples. An unidentified component with a retention time of 2.2 minutes under the GC conditions described earlier was also present in both fresh water and tap water.

The adsorption and desorption of DDT on the materials used to compound the aritificial sediments was briefly studied by batch experiments and with ^{14}C-labeled DDT. Experimental procedures and detailed results are given in Appendix A.

The following conclusions were reached:

a. DDT is quantitatively adsorbed on clay (see also Huang and Liao, 1970). The results of desorption experiments are inconclusive because a complete separation of clay and water may not have been achieved in these experiments.

b. DDT is poorly adsorbed by silica sand.

c. DDT is almost quantitatively adsorbed on the cereal used (complete separation between water and cereal may not have been achieved prior to the measurements). It appears that no subsequent desorption occurs in the first two hours when the tagged cereal is mixed with fresh water.

d. There is no apparent transfer of DDT from clay to cereal or vice versa.

It was inferred from these results that no measurable DDT would be present in interstitial water. Although interstitial water would have a lower redox potential and a lower dissolved-oxygen concentration than the water used in the adsorption-desorption experiments, these factors are not likely to directly affect the desorption and exchange of compounds like DDT. Possible biological mechanisms of transfer (through bacterial intermediates, for example) were not investigated.

Characterizations with regard to the organic material content of the sediments used in the finalized experiments were conducted by Mr. David Menzies, Research Assistant at the School of Oceanography, Oregon State University. The procedure that was followed consisted of keeping the samples frozen at -20° C until the time of processing, oven-drying them at 60° C for 3 days, weighing them in silver cups, and combusting them at 1100° C in an oxygen environment. Carbon and nitrogen determinations were carried out with a Carlo Erba 1100 CHN + O elemental analyzer, with standardizations against acetanilide. This method does not differentiate between organic carbon and carbonate carbon. Furthermore, the effect of sediment inhomogeneities is

magnified because of the small size of the aliquots that can be introduced into the instrument.

The results of the analyses are shown in Table 1. The following should be noted:

a. The organic carbon content of the Tubifex sediment is about what was expected. The composition of the cereal as given by the manufacturer was:

Protein	12.3%
Fat	4.5%
Carbohydrates	72.6%
Crude Fiber	0.9%
Ash	2.7%
Moisture	7.0%

The carbon content is calculated from these numbers to be about 40%. Thus a mixture of 1% pablum and 99% sand and clay should contain between 3 and 4 mg of carbon per gram of mixture. Therefore, the analysis does not appear to have a significant bias.

b. Based on the results from the Tubifex sediment the mixture of sterile sand, clay, and 0.1% pablum should contain about 0.35 mg C/g from the pablum. The remaining 0.2 mg or so of carbon per gram of sediment is contributed either by carbonaceous material remaining after "sterilization" at 400° C, or a small amount of carbonate, or both. The slightly higher C:N ratio indicates a considerable contribution from residual carbonaceous material.

c. The natural beach sand, used for the Nephtys experiments, is low in organic carbon, and therefore low in organic residue. The organic content of this sediment is estimated to be between 0.1 and 0.2%.

d. By contrast to the beach sand, the natural Capitella sediment contains about 5% organic material.

e. The results of replicate determinations in each set are within about ± 15% of the mean for three of the sediments, within ± 5% of the mean for one sediment, and within ± 24% of the mean for another sediment, with good agreement between the duplicate results from the natural beach sand.

TABLE 1

RESULTS OF CARBON AND NITROGEN ANALYSES
OF SEDIMENTS USED IN UPTAKE STUDIES

Sample	Replicate	Sample Wt.(mg)	Carbon (mg/g)	Nitrogen (mg/g)	C/N
Natural Capitella sediment Westport, WA	B	15.06	25.3	2.05	12.4
	B	13.83	28.4	2.28	12.5
	A	19.75	32.7	2.15	15.2
99.9% Natural Capitella sediment + 0.1% Pablum	B	19.40	24.4	2.27	10.8
	B	13.55	26.1	2.00	13.0
	A	19.26	24.2	2.48	9.8
98.9% Sterile Sand + 1% Clay + 0.1% Pablum	A	28.46	0.57	0.059	9.6
	A	35.79	0.50	0.054	9.2
	B	41.04	0.67	0.069	9.7
99% Natural Beach Sand + 1% Pablum	A	31.36	5.35	0.83	6.4
	A	36.88	4.93	0.67	7.3
	B	37.80	6.05	0.85	7.1
	B	41.55	4.70	0.67	7.1
Natural Beach Sand Tokeland, WA		47.21	0.60	0.069	8.7
		41.00	0.59	0.060	9.9
Artificial Tubifex sediment 98% Silica Sand +1% Clay +1% Pablum	A	39.31	3.59	0.41	8.9
	A	36.98	3.75	0.42	9.0
	B	38.55	3.42	0.42	8.1
	B	36.69	2.49	0.30	8.3

2. Preliminary Experiments with Capitella capitata

Although there was initial success in maintaining Capitella, subsequent experiments with Capitella resulted in high mortality. Originally, inadequate circulation of water in the aquaria was suspected as the cause. Shortening of the standpipes improved the circulation pattern to a satisfactory level, as indicated by dye tests. This improvement did not solve the problem, however. It appeared that the organic component of the artificial sediment may also have been a cause of the high (up to 100%) mortalities. In order to investigate this hypothesis, Capitella were placed in various combinations of sand, clay, and organic matter for six days. The mortalities ranged from 5 to 100% (Table 2).

TABLE 2

SURVIVAL OF CAPITELLA CAPITATA IN VARIOUS SEDIMENT COMPOSITIONS

Sediment Composition	Percent Survival
100% sterile beach sand	80
99% " " " , 1% clay	95
97.5% " " " , 2.5% clay	92.5
99% " " " , 1% aged cereal	20
99% " " " , 1% fish food	0

The sterile beach sand in these compositions was obtained from the intertidal zone where Nephtys was collected, and heated to 400° C. It was used in place of silica sand, because it was possible that the particle size of the sand might have an effect on survival. The results confirmed that Capitella is sensitive to the nature of the organic matter in the sediment.

Since the cereal and associated microbes in the artificial sediment were the only organic matter available to Capitella as a possible source of food, further experiments were conducted in which the mud that constituted the natural habitat of the worms was the major component of a tagged sediment. In this new set of experiments, separate clay and cereal additions, both tagged with ^{14}C-labeled

DDT, were made to the mud. Samples were collected at two, four, and seven days. The addition of cereal tended to decrease survival, particularly at the beginning, but after a few days there appeared to be little further change (Table 3). The occasional 100% mortality may have been unrelated to the cereal.

Since it appeared that survival was acceptable in a mud-1% clay-0.1% cereal mixture, a 22-day uptake study with Capitella was started on 6 December. However, during November the population of Capitella in the collection area began to decrease, and the specimens collected showed a high mortality even when left in their natural sediment. Because high mortality also occurred in the experimental containers, the specimens were judged to be unsatisfactory and the experiment was terminated.

3. Preliminary Experiments with Nephtys californiensis

Generally, no serious problems were encountered with Nephtys survival in the laboratory in either the natural sediment or in artificially compounded sediments. It was noted, however, that in artificial sediments the worms either behaved in a lethargic manner or attempted to escape from their containers. In addition, they did not appear to feed well on the artificial sediments. Additional evidence was obtained from an examination of the gut content of worms taken directly from the beach and of worms having been kept in artificial sediment. The gut of Nephtys maintained in the laboratory in artificial sediment for twelve days was almost empty, containing only some dark green filamentous algae. No sand, clay, or cereal was found in the gut and very little sand was found in the crop and gizzard. The guts of Nephtys collected from the beach were full and consisted of fragments of green algae, coccoid, and short rod bacteria. Sand grains were observed only in the crop.

The results of the first preliminary uptake study are shown in Table 4. Uptake was slow, but apparently uptake from clay (^{14}C data) was greater than uptake from cereal (^{3}H data). Relative to recoveries from tissue, inordinate amounts of DDT were found in the fecal samples, particularly ^{3}H-DDT. Since the worms were not observed to void their gut actively after having been transferred to sediment-free water, it was surmised that sediment adhering to the

TABLE 3

SURVIVAL OF CAPITELLA CAPITATA IN AND DDT UPTAKE FROM VARIOUS COMPOUNDED SEDIMENTS

A. SURVIVABILITY (Percent)

Exposure Time	Two Days		Four Days		Seven Days	
Sediment	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
98% mud, 2% clay (1)	85	60	75	75	70	100
99% mud, 1% cereal (2)	55	65	50	0	55	0
99.9% mud, 0.1% cereal (3)	100	70	65	55	55	65
control (100% mud)	95	-	85	-	100	-

B. UPTAKE (ng(DDT+DDD+DDE) /g tissue)

98% mud, 2% clay (1)	0.23	0.23	0.12	0.11	0.29	0.25
99% mud, 1% cereal (2)	0.23	0.40	0.14	-	0.5 (4)	0.78
99.9% mud 0.1% cereal (3)	0.28	0.33	0.27	-	0.43	0.32

(1) 30 ppb DDT.
(2) 30 ppb DDT - cereal aged for 24 hours.
(3) 1 ppm DDT - cereal aged for 8 days.
(4) A portion of this sample, estimated to be about 70%, was lost; the result given is an estimate.

TABLE 4

RESULTS OF THE FIRST PRELIMINARY STUDY OF DDT UPTAKE BY NEPHTYS*

Exposure Time hours	Tissue Samples DDT+DDD+DDE content (ng/g tissue)			Fecal Samples DDT+DDD+DDE content (ng total)		
	^{3}H-DDT	^{14}C-DDT	Total	^{3}H-DDT	^{14}C-DDT	Total
8	0.11	0.19	0.30	0.22	0.007	0.23
24	background	0.15	0.15	0.16	0.019	0.18
48	0.13	0.23	0.36	0.26	0.077	0.34
96	0.29	0.60	0.89	0.33	0.053	0.38

Exposure Time hours	Sediments DDT+DDD+DDE content (ng/g)			Sediments from control tank DDT+DDD+DDE content (ng/g)		
	^{3}H-DDT	^{14}C-DDT	Total	^{3}H-DDT	^{14}C-DDT	Total
8	12.9	24.7	37.6	17.6	--	--
24	22.9	20.6	43.5	17.1	--	--
48	14.2	26.7	40.9	26.5	--	--
96	7.6	12.4	20.0	20.0	--	--

*Sediment: 98% silica sand
1% clay and 30 ng ^{14}C labeled DDT per gram
1% aged cereal and 30 ng ^{3}H labeled DDT per gram

specimens was the major contributor to the activity that was measured in the fecal samples. No attempt was made in these samples to correct for any activity carried over from the sediments. In any event, it appeared that the accuracy of determining the pesticide content of fecal samples was low, because of unavoidable contamination by particles which were adhering to the worms until the worms were placed in water.

The sediments were analyzed to determine if loss of tracer occurred during the experiment. The data show a large variability of the DDT concentrations in the sediments. No consistent trend of the concentrations with time is evident. The variability of the data may be the result of sediment inhomogeneities, reflected in the aliquots taken for the analysis. It is quite possible, however, that the efficiencies of the DDT extraction from these sediments is much lower and more variable than the efficiency of the extraction of DDT from the sediments used in the blank experiments (Section IIG2).

A second experiment, covering about eight days, was conducted for the purpose of comparing uptake from sediments of various composition and also to compare uptake from and behavior in heat-treated and untreated natural sediment, with and without additions (Table 5). Virtually no uptake was found to have occurred from heat-treated or untreated natural sand with added tagged cereal. There was variable uptake from natural sand with added tagged clay, while uptake from tagged natural sediment (no additions) was approximately four times greater than from other sediments after the first day and twelve times greater by the end of the experiment. Nephtys appeared to burrow normally only in the tagged natural sediment.

Despite the fact that Nephtys is a selective feeder, there was uptake from clay. This was most likely related to the observation that some sediment was found in the crop and that some sediment remained adsorbed to the parapodia. Presumably some inorganic particulates are taken up in the crop to act as a grinding medium for the destruction of cell walls or organisms upon which the worms feed. In addition, most selective deposit feeders probably ingest substantial amounts of sediment, especially fines, at times.

On the basis of these observations a decision was made to use natural sand from where Nephtys was collected for future long-term studies.

TABLE 5

RESULTS OF SECOND PRELIMINARY STUDY OF DDT UPTAKE BY NEPHTYS

Sediment Evaluation

Sediment 1 : 99% sterilized beach sand (400°C, one hour)
1% aged cereal and 60 ppb ^{14}C-labeled DDT

Sediment 2 : 99% beach sand, untreated
1% clay and 60 ppb ^{14}C-labeled DDT

Sediment 3 : 99% beach sand, untreated
1% cereal and 60 ppb ^{14}C-labeled DDT

Sediment 4 : Beach sand, untreated, and 0.6 ppb ^{14}C-labeled DDT

Sediment	Exposure Time (hrs)	Sample Wt. (g)	Total DDT (ng)	ng DDT / g tissue
1	26	1.23	0.3	0.2
1	26	1.89	1.3	0.7
1	72	1.23	0.5	0.4
1	142	3.71	0.3	0.1
2	26	2.05	1.0	0.5
2	26	2.22	1.1	0.5
2	72	1.85	2.0	1.1
2	72	1.90	0.9	0.5
2	142	0.151	0.2	1.3
2	142	2.03	2.7	1.3
2	190	1.09	1.2	1.1
2	190	0.414	0.2	0.5
3	26	0.79	0.3	0.4
3	26	1.85	0.5	0.3
3	72	0.64	0.3	0.5

Table 5 (concluded)

Sediment	Exposure Time (hrs)	Sample Wt. (g)	Total DDT (ng)	ng DDT / g tissue
4	26	2.11	4.7	2.2
4	26	1.01	2.0	2.0
4	72	3.35	9.5	2.8
4	72	1.13	3.4	3.0
4	142	1.69	7.8	4.6
4	142	1.70	13.7	8.1
4	190	1.57	9.8	6.2
4	190	2.57	16.3	6.3

4. Preliminary Experiments with Tubifex tubifex

The first preliminary experiment lasted four days, with sampling at 1, 2, 3, and 4 days. The sediment consisted of 98% sand, 1% clay tagged with 30 ppb unlabeled DDT, and 1% cereal tagged with 30 ppb ^{14}C-labeled DDT. Some mortality was noted in one of the 3-day samples and 100% mortality occurred in one of the 4-day samples. Some uptake occurred during the experiment, to a level of about 0.3 to 0.4 ng/g of wet tissue.

In order to obtain higher precision in the measurements, particularly on tissue exposed for short times, it was deemed desirable to increase the DDT concentrations in the sediment admixtures to 100 ng/g. A 14-day study was undertaken to determine if this increase in the DDT level would adversely affect the organisms. Thus sediments were prepared in duplicate containing 98% sand, 1% clay tagged with unlabeled DDT at the 0, 30, 60, 100, and 150 ng/g levels, and 1% cereal with ^{14}C-labeled DDT, also at the 0, 30, 60, 100, and 150 ng/g levels. The 0 ng/g levels were used as controls. In addition to determining if any mortality had occurred, the organisms were analyzed for DDT after 7- and 14-day exposures.

There was 100% mortality in the 14-day samples containing 0 and 30 ng DDT/g of sediment, but virtually no mortality at the other concentrations. It appeared likely that the mortality at 14 days did not result from the exposure to DDT. Thus it was believed that experiments with 100 ng DDT/g of sediment could be carried out without causing acute toxicity. It is further noted that in the later 127-day experiment no instances of unexpected mortality occurred.

The results of the analyses are plotted in Figure 4. The vertical lines connect the data points from duplicate runs, with the actual points shown being the averages of these duplicates. Linear regression analysis for a $y = bx$ relationship showed no statistically significant difference at the 95% confidence level between the b-values at 7 days and at 14 days. The line drawn considers all points and has a slope of 0.021 (ng/g tissue)/(ng/g cereal). The concentrations of DDT in the organisms are approximately proportional to the labeled DDT added to the sediment.*

*It will be shown later that dynamic equilibrium (regulation) will occur after about 20 - 30 days. The validity of the conclusion reached here should not be extrapolated to the equilibrium concentrations without further proof.

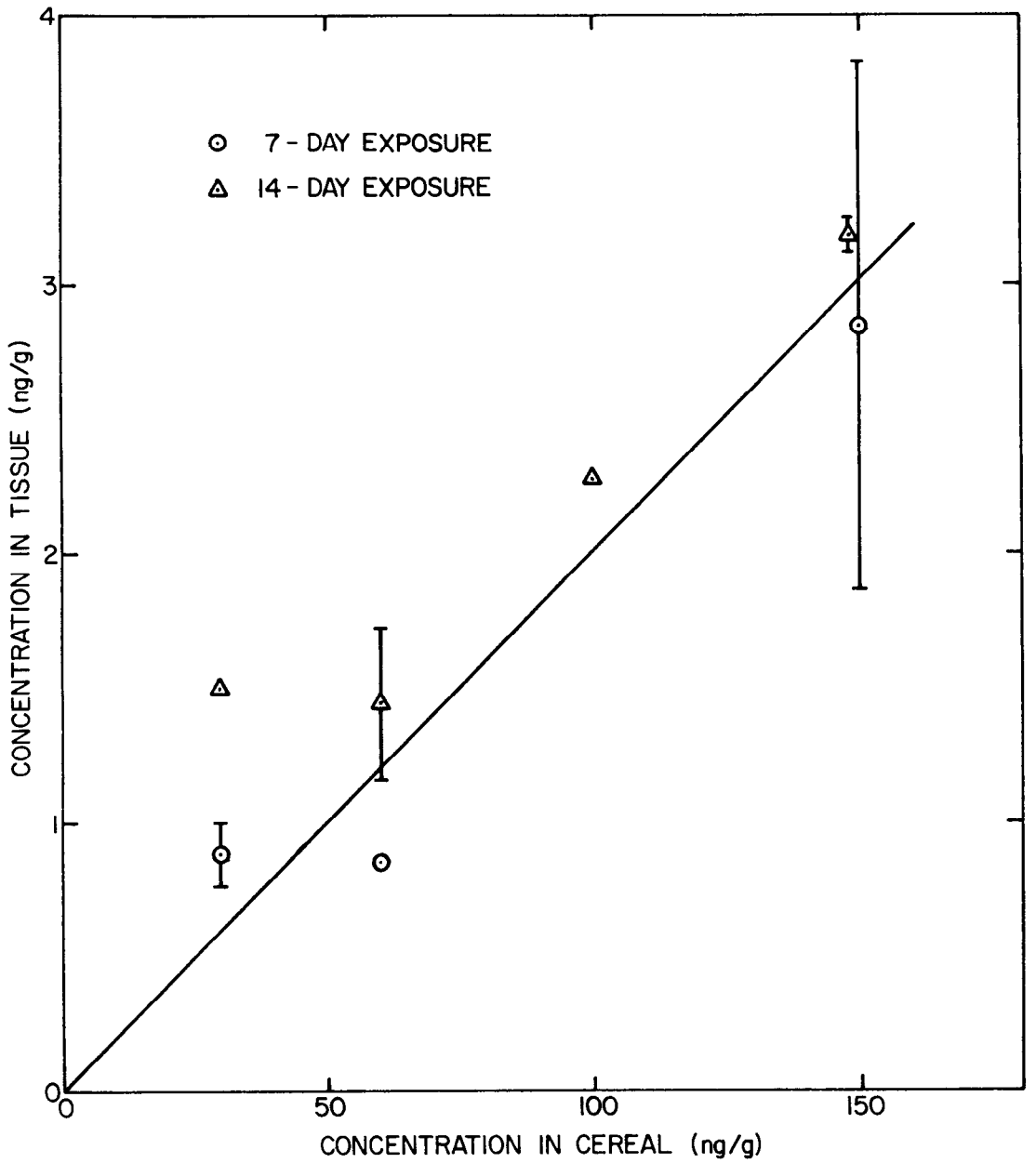

Figure 4 DDT concentrations in Tubifex tubifex after 7 and 14 days exposure to sediments containing different amounts of DDT

A longer-term experiment was run for about 23 days at the higher DDT level. The sediment composition was as follows:

98% silica sand
1% 24-hr aged cereal with 100 ng ^{14}C-labeled DDT/g cereal
1% clay with 100 ng ^{3}H - labeled DDT/g clay

The results are shown in Table B1 (Appendix B). The data points plotted in Figure 5 represent the averages of the two replicates. Definitive conclusions cannot be drawn because of the scatter of the data and the limited duration of the experiment. It is noted, however, that some differences may exist between the uptake from clay and the uptake from cereal, as judged from the behavior of the $^{14}C/^{3}H$ ratio with time.

B. Long-Term Experiments in Compounded Sediments

1. Uptake of DDT by Tubifex tubifex

A 52-day experiment was completed with Tubifex tubifex in sediment of the following composition:

98% silica sand
1% 24-hr aged cereal with 100 ng ^{3}H-labeled DDT/g cereal
1% clay with 100 ng ^{14}C-labeled DDT/g clay

The experimental data are shown in Table B2 (Appendix B) and are plotted in Figure 6. The points in this figure, as well as in Figures 7 and 8 (see below), have been correlated in an approximate manner by means of curves whose meaning is discussed and whose parameters are calculated in Section IV.

The data from the 23-day experiment and the first 22 days of the 52-day experiment are generally within a factor of 2 of each other or better. Thus similar conclusions are reached from both experiments with regard to uptake of DDT during the first 23 days: some difference in uptake from clay and from cereal is indicated by the activity ratios ($^{3}H/^{14}C$ - note the reversal of the labels compared to the 23-day experiment). During the first 7 - 10 days uptake from the cereal appears to be faster than uptake from the clay, with the differences in the rates decreasing rather rapidly, however. After 10 days, the ratio of DDT in the tissue derived from the cereal to that derived from the clay remains more or less constant at about 1.3 (average). (In the 23-day

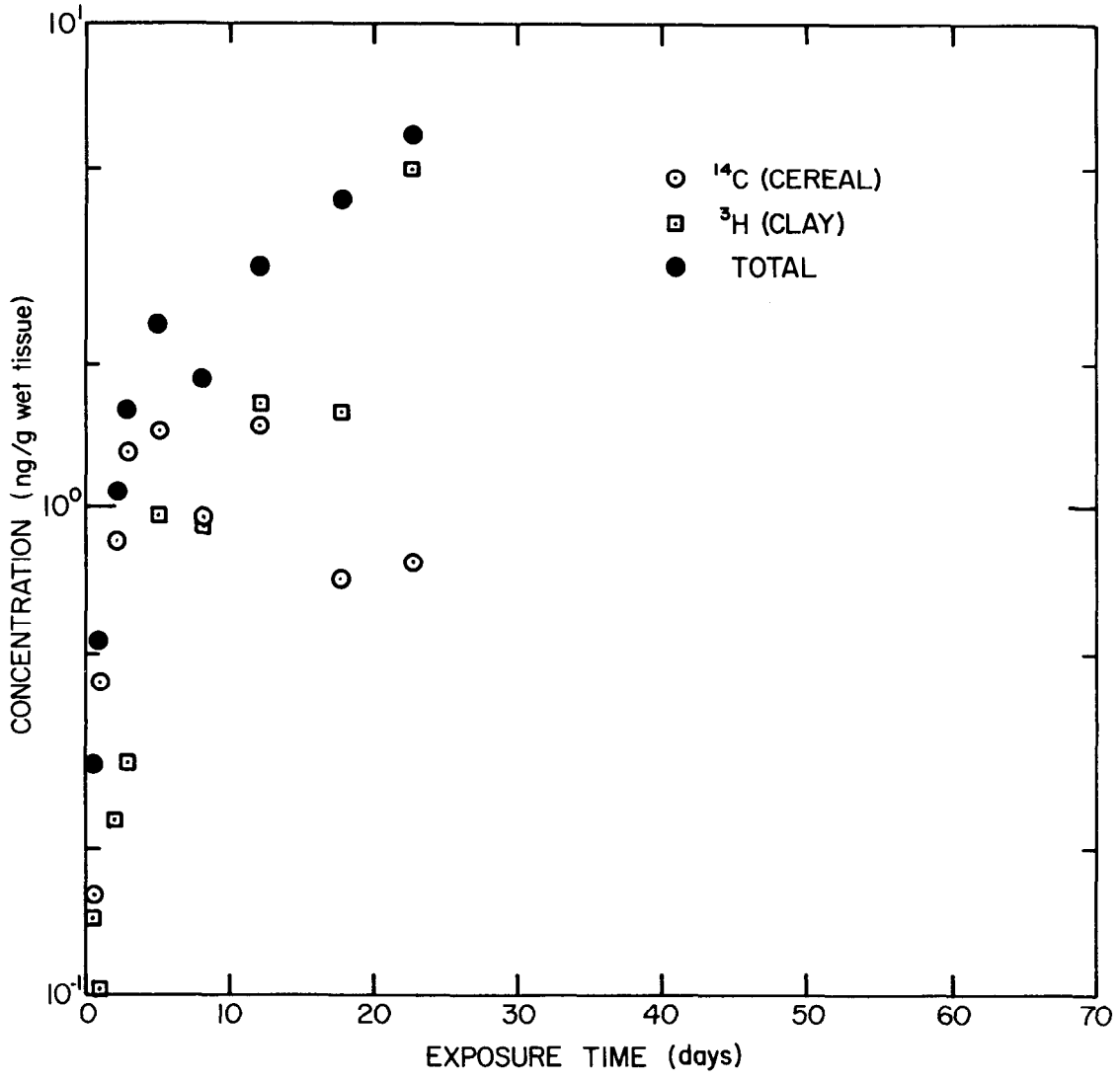

Figure 5 Uptake of DDT from artificial sediment by Tubifex tubifex (23-day experiment)

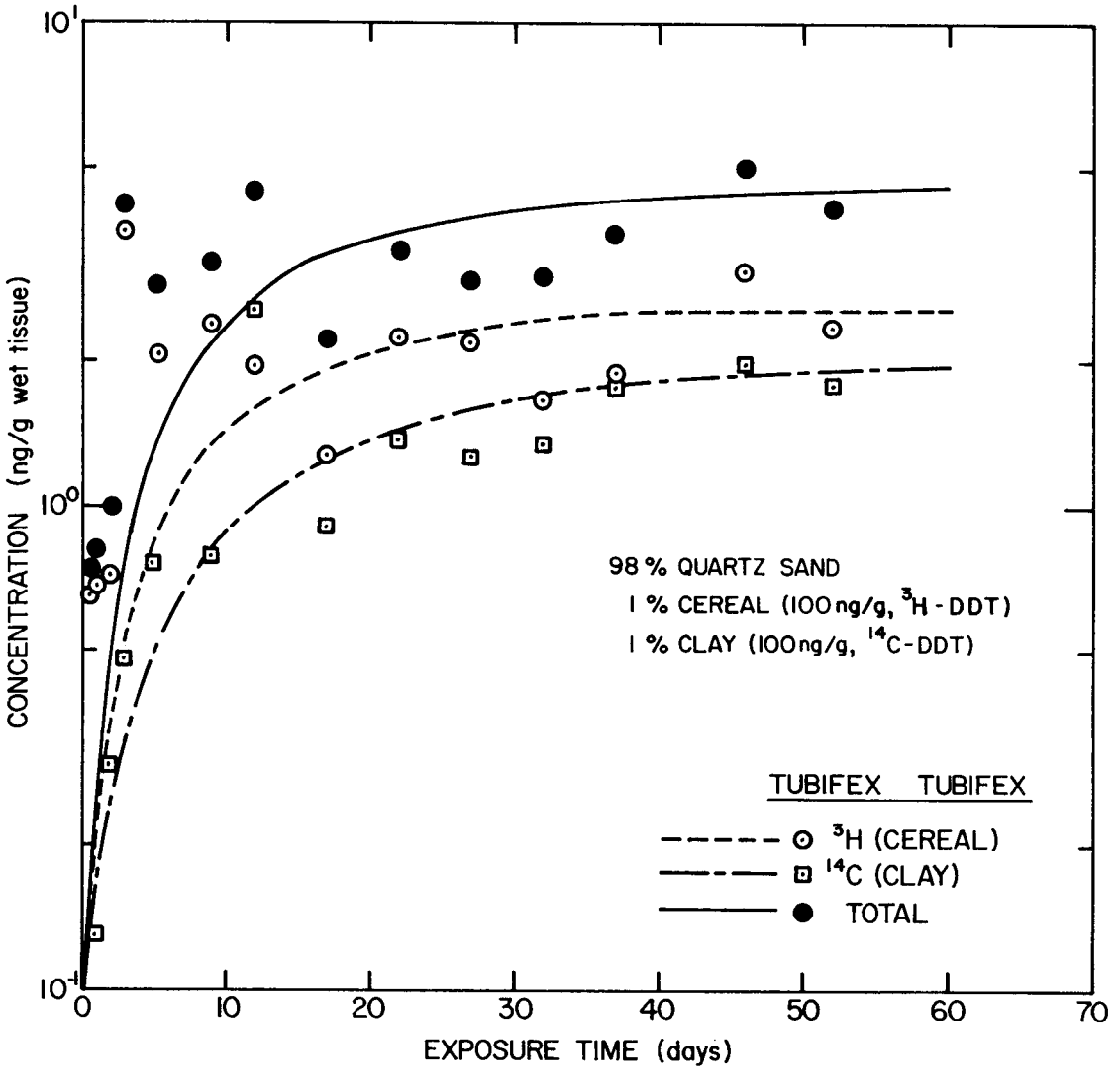

Figure 6 Uptake of DDT (and metabolites) from artificial sediment by Tubifex tubifex (52-day experiment)

experiment this ratio continues to decrease.) Whether or not one can draw the conclusion that DDT is a little more available from organic matter than from inorganic matter is problematic because of the artificiality of the organic component of our sediment.

The DDT (and metabolite) content of the sediments was determined on the 17-day and the 32-day replicates. The results are shown in Table B3 (Appendix B). The data indicate that no DDT was lost from the sediments between 17 and 32 days. The concentrations found were only about one half of those expected. This observation raises the question whether there was a significant loss of DDT during the first 17 days, or if the recovery of DDT during the analysis was less than the recovery obtained during the blank experiments (Section IIIA). This question is addressed in some detail below in the discussion of the results with Capitella, where it is argued that low analytical recovery is the reason for the lower-than-expected DDT concentrations found.

2. Uptake of DDT by Capitella capitata

A 55-day run was completed with Capitella capitata in sediment of the following composition:

99.8% beach sand (baked at 400° C)
0.1% 24-hr aged cereal with 1000 ng ^{14}C-labeled DDT/g cereal
0.1% clay with 1000 ng ^{3}H-labeled DDT/g clay

The experimental data are shown in Table B4 (Appendix B) and are plotted in Figure 7. There is little difference between DDT-uptake from clay and from cereal. In addition, it appears that some degree of internal control of DDT concentrations is reached after 30 - 35 days. In contrast to the Tubifex data, uptake from clay may initially be a little more rapid than uptake from cereal. However, the ratio of DDT in the tissue from cereal to that from clay is eventually quite comparable to what was found in Tubifex. In Capitella the overall concentration factor, relative to the entire sediment, is considerably higher than in Tubifex.

The concentrations of DDT measured in the sediment are shown in Table B5 (Appendix B). They should be compared to an initial concentration of 1 ng each of ^{14}C-labeled DDT (on cereal) and of ^{3}H-labeled DDT (on clay) per gram of sediment. The variability of the concentrations found is rather

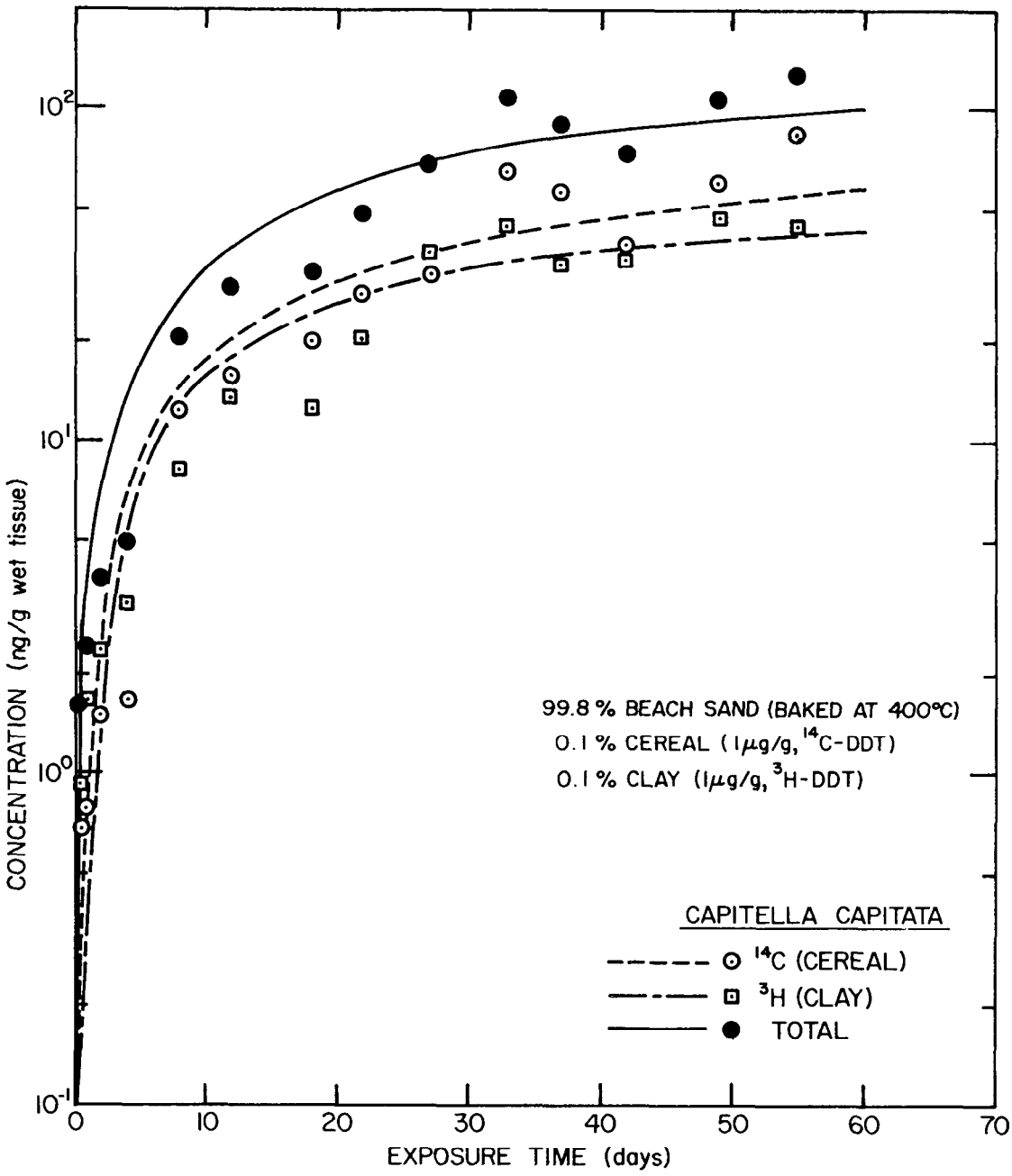

Figure 7 Uptake of DDT (and metabolites) from artificial sediment by Capitella capitata

large, and the data are not clearly interpretable. The ^{14}C data may suggest a loss of DDT from cereal with time, but a similar loss of DDT from clay is not evident from the ^{3}H data.

It appears that after half-a-day the DDT levels in the sediments have decreased by more than 50%. We believe most or all of this apparent decrease not to be real, but to be the result of an extraction efficiency lower than indicated by our blank experiments described in Section II2. This judgment is based on the following:

As a result of the manner in which the aliquots from the sediments are obtained for extraction and further DDT analysis, the analyzed portions are close to representative of the entire sediment. Any loss of DDT during the uptake experiments would primarily occur in the upper portions of the sediments. Thus an actual reduction of the average DDT-concentration of the sediment by 50% or more during the first twelve hours of the experiment could only have occurred if these had been a very rapid depletion of DDT to a significant depth. However, DDT depletion would occur primarily by diffusion into the water above the sediment, taking into account also the effect of the burrowing action of the organisms. Since diffusional processes are relatively slow, it appears that the low DDT levels found cannot be explained adequately by assuming a loss of DDT to the water.

An attempt was made to confirm the occurrence of a low extraction efficiency. Fresh aliquots of sediments were taken from the containers removed after 18.2 days (both replicates) and after 54.9 days (one replicate). The labeled DDT concentrations were determined in these aliquots in the standard manner, the results having been included in Table B5. The residues left after extraction were treated under reflux with concentrated nitric acid to destroy the DDT (and other organic matter). The ^{14}C, being converted to $^{14}CO_2$, is lost, but the ^{3}H remains behind as tritiated water. After cooling the suspension was filtered and 100-μl aliquots of the approximately 20 ml of liquid were counted in a liquid scintillation counter. The results were inconclusive. No significant counts above background were obtained.

The reproducibility of the DDT determinations may be estimated from the data at 18.2 days and at 54.9 days and is about $\pm$10%. With due consideration of this reproducibility and of the possibility of low recoveries, the ^{14}C data

suggest a loss of DDT from cereal with time, by perhaps about one-third during 55 days. No such loss from clay is suggested by the ^{3}H data.

3. Uptake of DDT by Nephtys californiensis

A 127-day experiment was completed with Nephtys californiensis in sediment of the following composition:

99% natural sediment
1% natural sediment with 60 ng ^{14}C-labeled DDT/g sediment

A second, 23-day experiment was run with the 1% tagged natural sediment replaced by 1% sterile beach sand that was tagged with 60 ng ^{14}C-labeled DDT per gram. The purpose of this experiment was to observe any uptake of DDT from just the inorganic component, since both organic and inorganic components were tagged in the 127-day experiment.

The data from the 127-day experiment (Table B6, Appendix B) and from the 23-day experiment (Table B7, Appendix B) indicated that some DDT was taken up and that some internal control occurred after about 70 days. The concentration factor with respect to the total sediment was less than 10.

The 23-day experiment, with tagged clay, was run to determine if any portion of the observed uptake in the long-term experiment was from clay. The results of the preliminary experiments had indicated the ability of Nephtys californiensis to take up DDT adsorbed on clay. The procedure used to tag the sediment for the long-term experiment did not allow a distinction to be made between tags on the organic and on the inorganic component. The results (Table B7, Appendix B, and Figure 8) confirm the ability of Nephtys californiensis to assimilate at least some DDT from clay, although complete removal of all organic matter (organic carbon) by baking the sediment at 400° C was not proven. Indeed, a comparison of the data from the two experiments suggests that when the natural sediment was tagged, the tag may well have gone mostly on a clay or other fine-particulate component, as the carbon analysis showed approximately 1% of organic material to be present.

The results of sediment and effluent water analyses are shown in Table B8 (Appendix B). The theoretical concentration in the sediment was about 0.60 ng DDT per gram of sediment, in the long-term run as well as in

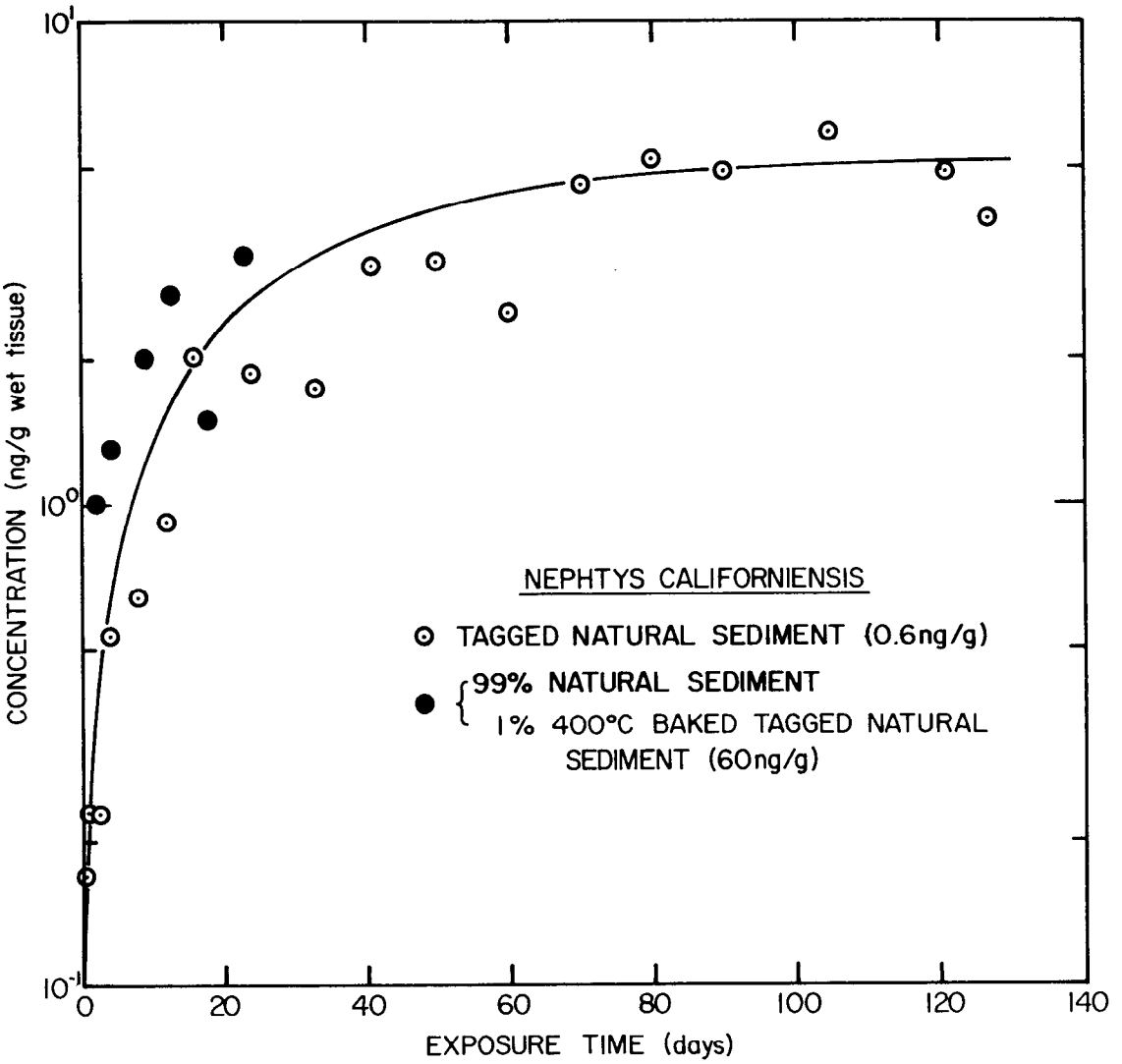

Figure 8 Uptake of DDT (and metabolites) from tagged natural sediment by Neptys californiensis

the run where labeled clay was added to the natural sediment. Recoveries appear to be about 40%, as before. However, the recovery from the sediment with the labeled clay is a little higher, approximately 60%. Loss of DDT from the sediment is not evident.

None of the water samples analyzed showed labeled DDT concentrations statistically different from background.

C. Metabolite Studies

A number of tissue and sediment samples were analyzed for DDT, DDD, and DDE with separation by thin-layer chromatography as the key analytical step. Table B9 (Appendix B) shows the results, expressed as the relative fraction of each of these three compounds and based on the total ^{3}H or ^{14}C recovered. The fraction of total remaining DDT, calculated by averaging the means of the ^{14}C-labeled DDT fractions and the ^{3}H-labeled DDT fractions, is plotted against time in Figure 9 through 11. The straight lines drawn through the points are visual estimates.

The data show the conversion of DDT and DDD to DDE to be extremely slow, if proceeding at all, the levels being at or near the detection limit. The only exception appears to be the Tubifex tissue, for which the data are very erratic, however. This particular set of data was obtained early in the project, whereas all other metabolite data shown were obtained in a more systematic manner. Thus the rates and degrees of DDT conversion in Tubifex tissue need confirmation.

In the Nephtys experiment there did not appear to be any important difference between the DDT-DDD composition of the tissue and the sediment. In the Capitella experiment this may also be true, although the few data obtained may indicate a slightly lower DDT fraction in the tissue compared to the sediment. The uncertainty in the Tubifex tissue data precludes any adequate comparisons being made of the tissue and sediment data from the Tubifex experiment.

It appears that degradation of DDT to DDT may be described with sufficient accuracy to represent the data as first-order reactions in the sediment. For the Tubifex and the Nephtys experiments, the rate constant is about $0.004\ d^{-1}$. For the Capitella experiment the rate constant is about $0.014\ d^{-1}$.

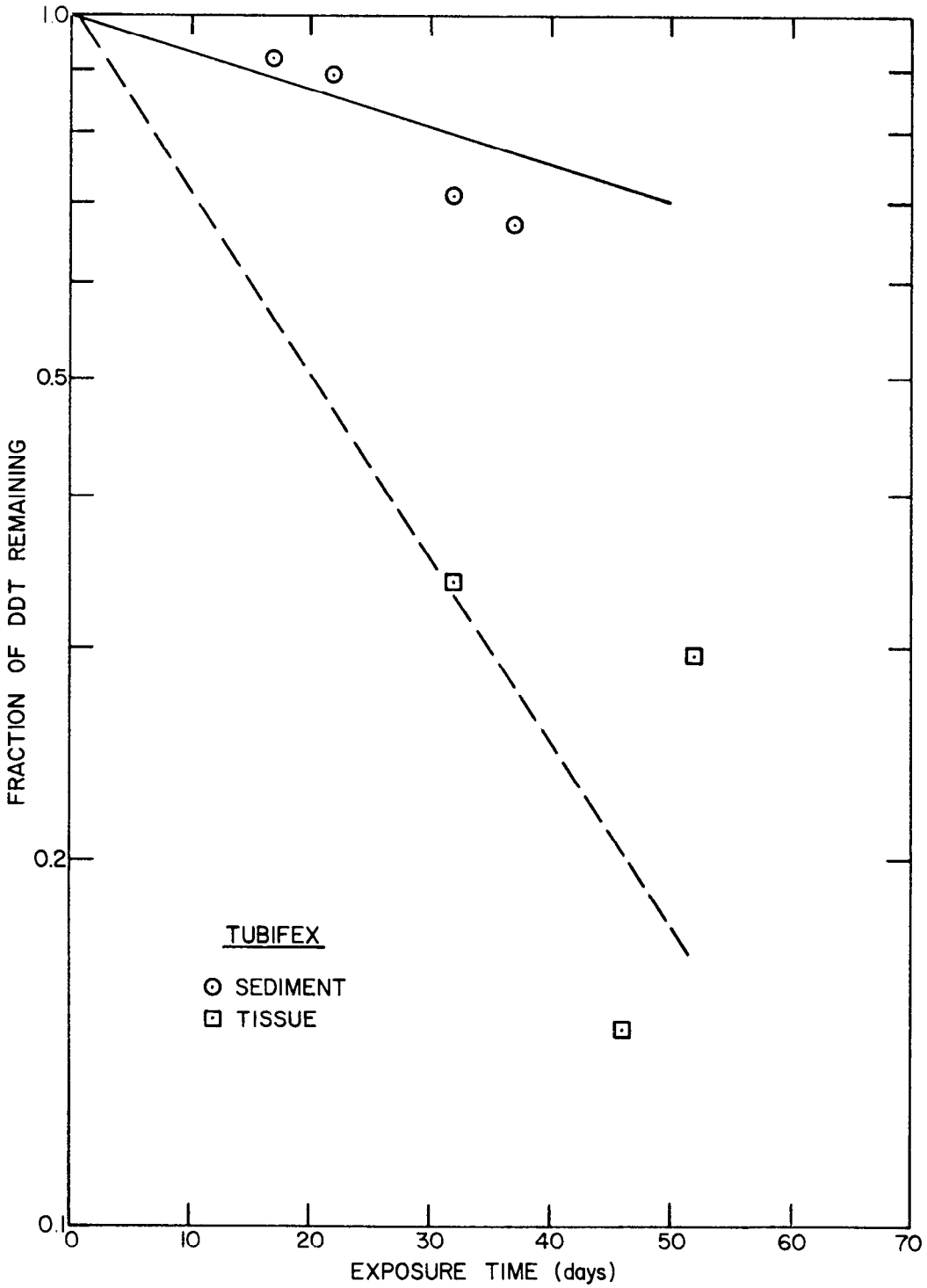

Figure 9 Metabolism of DDT in sediment and in tissue during the uptake experiment of DDT by Tubifex tubifex

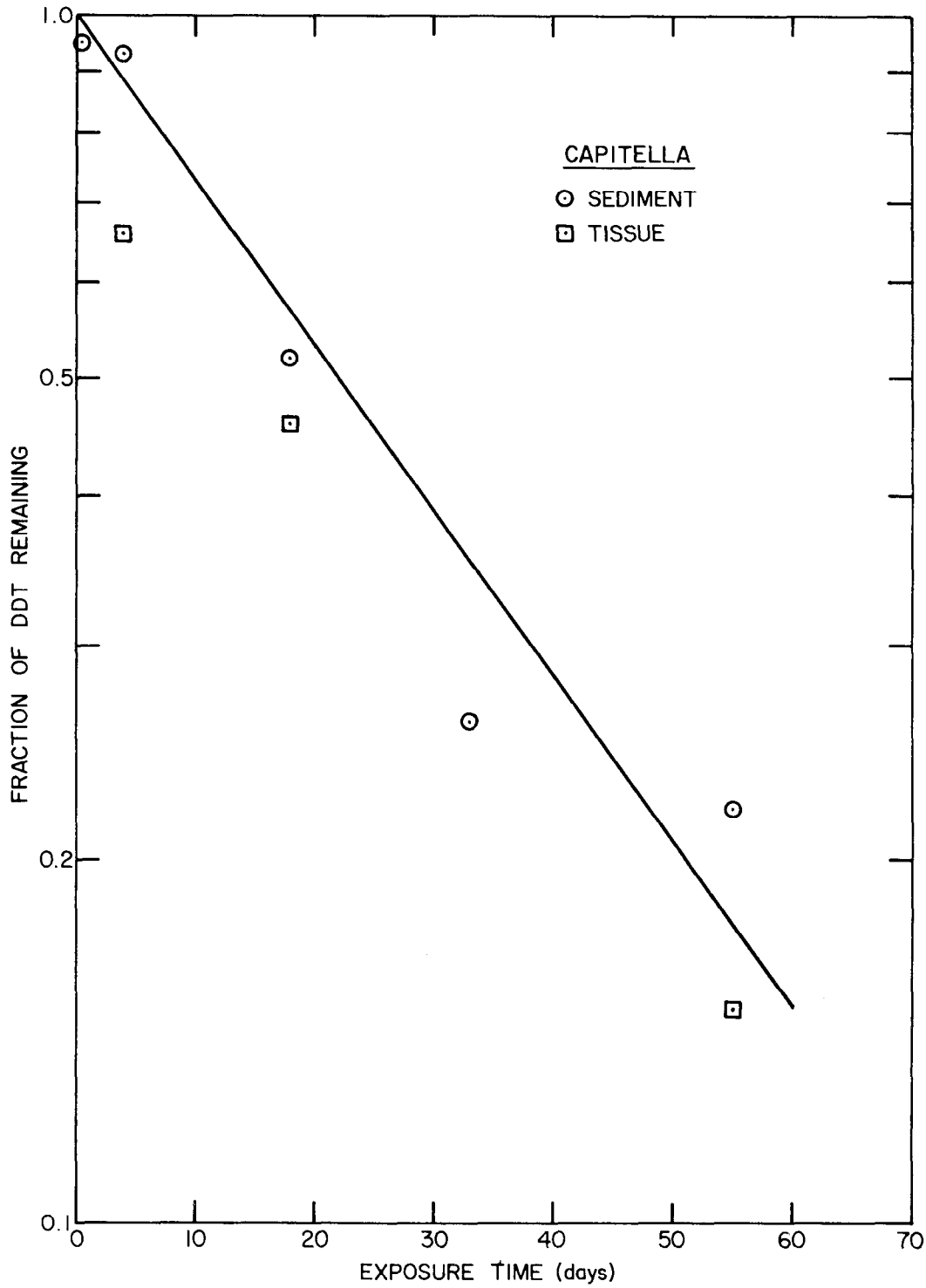

Figure 10 Metabolism of DDT in sediment and in tissue during the uptake experiment of DDT by Capitella capitata

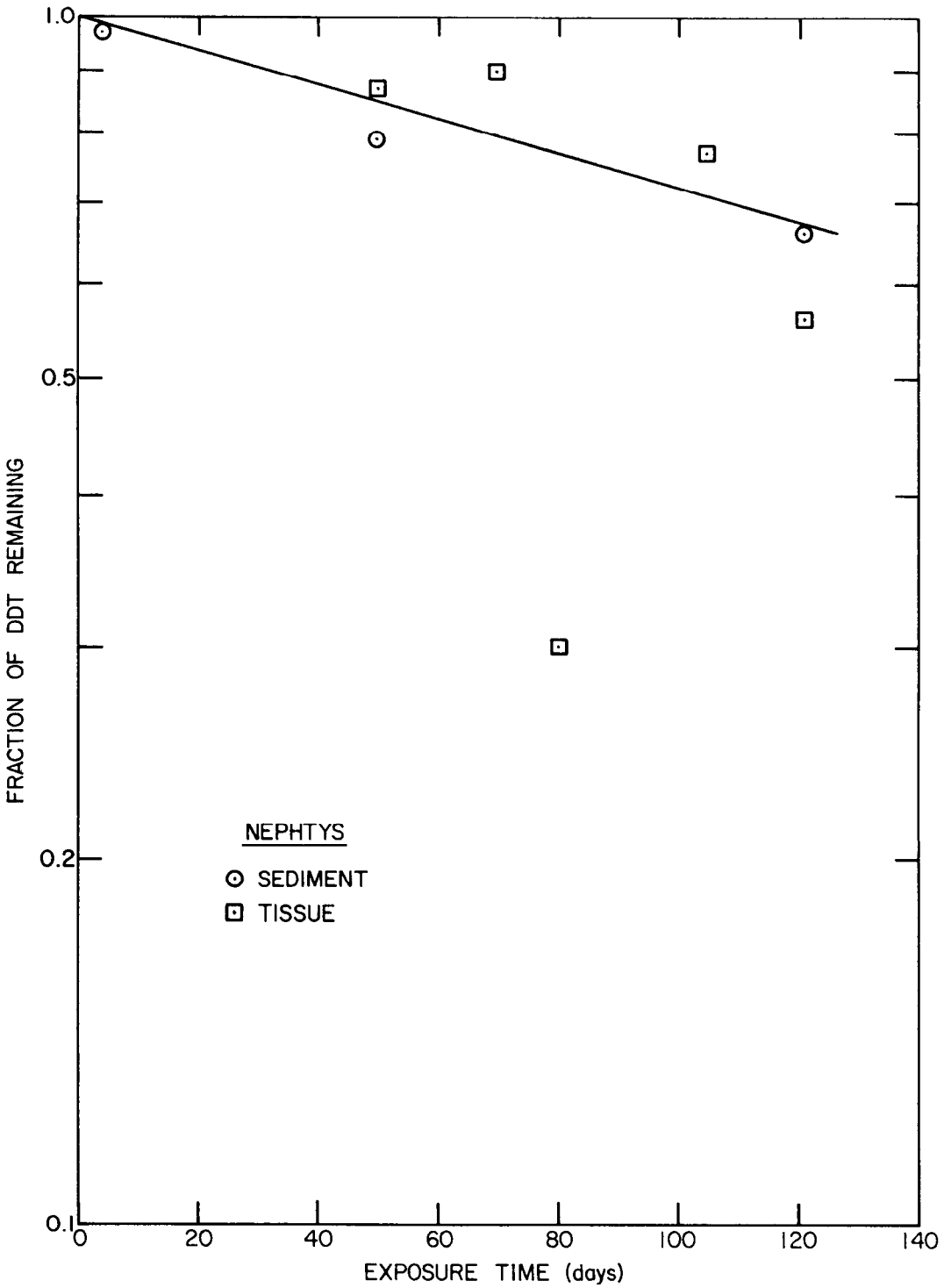

Figure 11 Metabolism of DDT in sediment and in tissue during the uptake experiment of DDT by Nephtys californiensis

IV. DISCUSSION

The experiments that have been described and their results must be viewed in the light of the original program and its objectives as discussed in the introduction. The basic questions that were attempted to be answered were:

a. Do selective and nonselective marine and freshwater deposit feeders take up DDT and its degradation products, DDD and DDE?

b. If the answer to the previous question is positive, are DDT and its degradation products available from interstitial water, from ingested detritus (for selective feeders), or from detritus plus clay particles?

c. If uptake occurs, are uptake and accumulation controlled by internal regulation?

These questions are primarily of a qualitative nature. Hence the approach was a compromise between an entirely qualitative survey and a program designed to provide detailed mechanistic and kinetic information. Thus the number of replications was limited to two, and the sampling frequency also was limited. No attempt was made at all to determine the kinetics of elimination of DDT and its degradation products.

In addition to limitations placed upon the conclusions by the extent of the program itself, some limitations are also the result of the nature of the data obtained, particularly the degree of agreement between replicates, and the question regarding the recoveries.

Recoveries (or extraction efficiencies) from tissue have not been determined separately during the program. However, previous experience at LFE Environmental has indicated recoveries of pesticides including DDT of about 90% from tissue samples. Although it may be argued that in the subject samples DDT may have been incorporated differently than in other tissues in such a way as to reduce the recovery, there appears to be no reason why this would have been the case.

The concentrations measured in the sediments are generally between 25 and 50% of the starting concentrations. Almost the entire decrease, as measured, occurs during the first 12 hours. There is no clear evidence that a further decrease occurs after the first half-day. None of the concentrations

of DDT found in the water samples analyzed were significantly different from zero. These low concentrations in the sediment cause a problem in the interpretation of the data. No sediment samples taken prior to the start of the experiment or immediately after preparation were analyzed for the added DDT. Therefore, one can only speculate about the reasons for the decrease in the measured concentrations. It is the authors' opinion that either the results of the sediment analyses do not reflect the actual concentrations, or else that perhaps a consistent error has been made in the starting concentrations. This latter possibility may virtually be ruled out because the procedures were frequently checked. The reasons for the opinion that the recoveries rather than the concentrations are low have been discussed in Section III: the rate of depletion of DDT in the sediment during the first few hours of the experiment would have to have been unreasonably high, diffusional transfer of interstitial water being the mechanism by means of which depletion could occur. In addition, however, adsorption experiments showed that the adsorption equilibria of DDT between clay and water and between cereal and water are so far on the side of adsorption that no measurable concentrations of DDT in the water were found. Furthermore, no measurable transfer of DDT between clay and cereal was found to occur during these preliminary experiments.

Because of these same results it appeared reasonable to assume that interstitial water would not play a major role in contributing to any accumulation of DDT by the organisms. Thus no separations of interstitial water were made, and, therefore, no separate determinations of DDT concentrations in interstitial water. It is also noted that such separations would have been very difficult because of the near-colloidal nature of some of the constituents of the prepared sediments.

The plan of the experiments included a step whereby the organisms were placed in clean water to give them an opportunity to void their guts, so that the true tissue concentration would be measured without any contributions from the sediments. Since it was feared that long residence times of the organisms in the water might cause elimination of DDT and its degradation products from the tissues themselves, a residence time of one hour was decided upon as a reasonable compromise. It was noticed, however, that many of the organisms remained rather inactive after having been placed in the water so that complete voiding of the guts probably did not occur. Thus the DDT-concentration data

for the organisms represent both assimilated DDT as well as DDT in the gut that could have been passed when the gut was emptied.

The water with the solid material was saved after removal of the organisms, and a few of these samples were analyzed. The analyses were not successful, however, because complete removal of sediment particles from the exterior of the organisms after they were taken from the sediment was not possible without damaging the organisms.

With the possible exception of Tubifex tissue, the amounts of DDE found in the sediments and in the organisms were all at or below the detection limit. Thus a consistent increase of DDE concentrations with time was not noted. It is concluded that the rates of degradation of DDT and DDD to DDE were very slow, with the possible exception of Tubifex. The data for Tubifex are rather erratic, however, and do not allow any conclusions regarding the degradation of DDT to be drawn. By contrast, DDT appeared to be subject to continuing degradation to DDD in all sediments and tissues during the course of the experiments.

The rates of increase of DDD in the sediments and in the tissues were about the same in each experiment; however, these results suggest that degradation of DDT occurred primarily in the sediments. The experimental design and consequently the data for Capitella and Nephtys did not allow a determination of whether the organisms were taking up DDD from the sediments, degrading DDT in their tissues, or both.

Returning to the original questions posed at the onset of this discussion:

a. The selective and nonselective marine and freshwater deposit feeders studied in this work take up DDT and presumably also DDD. Further degradation of DDT and (or) DDD to DDE never occurred far enough in the sediments to draw any conclusions regarding uptake of DDE.

b. In the experimental system used DDT is available from detritus and clay particles, certainly taken together and probably separately, for both the selective and the nonselective feeders. Availability from interstitial water requires the presence of DDT (and DDD) in interstitial water, and this presence could not be shown to occur. Hence the question regarding availability from interstitial water may be academic. However, the mechanism

by which the organisms take up DDT has not been elucidated, and has not even been addressed.

c. The concentrations in the organisms appear to reach a constant level in Tubifex tubifex after about 30 - 35 days, in Capitella capitata after about the same time, and in Nephtys californiensis after about 70 - 80 days. Hence some degree of internal regulation of DDT concentrations appears to occur. Regulation is defined here to mean control by way of an internal active or passive mechanism. The specific mechanism of control was not investigated as part of this study. However, possible mechanisms include regulation through homeostatic processes and chemical equilibrium. Homeostatic processes enable an organism to maintain its internal environment within narrow limits permitting survival and reproduction. Homeostatic responses to changes in the organism's external environment are adaptive responses and involve feedback controls. On the other hand, chemical equilibrium is based on differences in the solubility of a substance in various media and result in the substance being concentrated in the medium in which it is most soluble.

An attempt was made to derive an analytical description of the uptake data. Neither the design of the experiments nor the data that have been obtained allow the proper evaluation of any detailed models for the dynamics of DDT and its degradation products in the experimental systems used. Thus the description of the uptake data will be based upon a simple, two-compartment model consisting of the sediment and the organisms as the compartments. The sediment is considered as a constant source, because the amounts of DDT and DDD in the organisms are small compared to those in the sediments, even compared to upper third or half of the sediments where the organisms were located. Since the DDD/DDT ratios in the tissues and in the sediments were about the same at any one time, DDT and DDD are treated together as a single component. The kinetics of transfer between the sediment and the organisms are assumed to be first-order and uptake from the organic and inorganic components are assumed to be independent. From a kinetic standpoint a dynamic equilibrium is assumed to be established at large values of the time.

The differential equations that describe this system are:

$$\frac{dc_s}{dt} = 0 \qquad (1)$$

$$\frac{dc_o}{dt} = k_1 c_s - k_2 c_o \qquad (2)$$

where c_s and c_o are the DDT + DDD concentrations in the sediment and in the organisms, respectively, and k_1 and k_2 are rate constants. The boundary conditions are: $c_o = 0$ at $t = 0$, and $c_o = c_o, \infty$ at $t \to \infty$ and $c_o = c_o$ as $t \to \infty$. For Capitella and for Tubifex equations (1) and (2) are to be solved separately for the ^{14}C and ^{3}H data representing uptake from the organic and inorganic components of the sediments.

The solution of equations (1) and (2) is:

$$c_o = \frac{k_1}{k_2} c_s(1 - e^{-k_2 t}) \qquad (3)$$

The parameters in equation (3) may be obtained by means of the application of a least-squares fitting routine either by hand calculations or by a digital computer. The unusually simple form of equation (3) lends itself to hand calculations. For long exposure times, that is, as $t \to \infty$, $c_o \to \frac{k_1}{k_2} c_s$, the steady-state concentration. With $\frac{k_1}{k_2} c_s = c'$ thus obtained, equation (3) can be linearized as

$$\ln \frac{c' - c_o}{c'} = -k_2 t \qquad (4)$$

and k_2 can be estimated by a linear least-squares procedure. In order to get a starting value for c' it is more convenient to specify that $e^{-\lambda t}$ be small, say, ≤ 0.1, for asymptotic behavior, to avoid the useless result that $k_2 \simeq \infty /t$. Then $c_o \geq 0.9\, c'$, and $k_2 t \geq 2.3$ or $k_2 \geq 2.3/t$ for some t at which c_o approaches c'. The values of c' and of k_2 thus obtained are approximations to the actual values. The best-fit values can then be obtained by application of the normal equations minimizing the values of $(c_o, \text{measured} - c_o, \text{calculated})^2$.

The best values for the rate constants thus obtained are shown in Table 6. The curves calculated from these numbers are those drawn in Figures 6 through 8.

TABLE 6

BEST VALUES OF RATE CONSTANTS AND STEADY-STATE CONCENTRATIONS FOR DDT UPTAKE BY BENTHIC ORGANISMS

Organism	Tagged Substrate	k_1 (ng/g/day)	k_2 (ng/g/day)	Steady-State Concentration (ng/g)
Tubifex tubifex	Clay	0.12	0.058	2.0
id.	Cereal	0.21	0.08	2.6
Capitella capitata	Clay	1.9	0.040	47.0
id.	Cereal	2.0	0.028	70.0
Nephtys californiensis	Beach sand	0.26	0.029	5.3

For short times, typically less than two weeks, the predicted curves tend to overestimate the measured values. Mathematically this implies that c', or k_2, or both are too large, but the asymptotic behavior restricts the lower values of both c' and k_2. It may be concluded that the mathematical description is too simple, and that equation (3) with the values of the constants shown in Table 6 is a convenient approximation to the uptake behavior of the organisms.

The conclusion from our experiments that regulation (as defined earlier) occurs is of particular importance for the development of disposal criteria. Rather than accumulation of DDT (and its metabolites) continuing almost indefinitely when annelids are exposed to a constant source, a steady state is reached after some period of time. Consequently a concentration factor, or bioaccumulation factor, can be defined for the interaction of annelids with DDT in sediments. A brief discussion of bioaccumulation factors (with special reference to radionuclides, however) is given by Vanderploeg et al. (1975). The definition of the bioaccumulation factor of an organism or tissue is defined as the steady-state ratio of the DDT concentration in the organism or tissue to that in sediment:

$$BF(D)_i = [D]_i/[D]_S \qquad (5)$$

where $BF(D)_i$ = bioaccumulation factor for DDT in organism or tissue i

$[D]_i$ = DDT concentration (ng/g wet tissue) in organism or tissue i

$[D]_S$ = DDT concentration (ng/g) in sediment, a constant

The data from the experiments indicated that the bioaccumulation factor for DDT was constant regardless of the DDT concentration in the sediment within the range of concentrations employed. A more general statement about this relationship cannot be made as the toxicity experiments with Tubifex were terminated before a steady state had been reached. Furthermore, similar experiments with Capitella or Nephtys were not conducted.

If it is reasonable to define bioaccumulation factors, these factors for the species and experimental conditions used were:

about 2 for Tubifex tubifex
about 50 for Capitella capitata
about 8 for Nephtys californiensis

The validity of these bioaccumulation factors is by no means certain, and extrapolation for use with sediments in natural waters is not justified without confirmation. For example, the bioaccumulation factor is affected by the availability of DDT to the organisms. Thus a more precise definition of this quantity would employ the concentration of available DDT rather than the concentration of all DDT. Although it has been shown that at least some of the DDT is available from clay and from organic matter, no estimate can be made of the actual fraction of DDT that is available to the organisms. It is quite possible, for example, that not all clay-adsorbed DDT is available as there may be some differences between the adsorption sites. The mechanism of transfer must be investigated before more definitive statements about availability can be made.

In some contaminated sediments the sizes of some of the particles or components of detritus may be too large for some species of infauna to ingest. Although DDT adsorbed on this material could be considered as unavailable to those species, over time this DDT or its degradation products might be made available through bacterial intermediaries or decomposition of the large particles. Therefore, in the determination of bioaccumulation factors from contaminated sediments in natural waters the particle-size distribution may have to be taken into account to make valid generalizations possible. That is, the DDT concentration should be determined not only in the sediment as a whole, but also as a function of the particle size. Alternatively and perhaps more practically, a determination of the DDT concentration in, for example, the <44-μm (325 mesh size) fraction of the sediment may suffice.

The question may be raised whether the bioaccumulation factor should be defined relative to the total sediment, to only that fraction which contains the contaminant (DDT in thise case), or to the available fraction of the DDT. In the experiments the contaminated fraction is defined by the way the sediment was prepared. This fraction is 0.01 for the sediments used with Tubifex and with Nephtys, but 0.001 for the sediment used with Capitella. If the bioaccumulation factors are related to this fraction only, they are calculated to be 0.02 for Tubifex tubifex, 0.05 for Capitella capitata, and 0.08 for Nephtys californiensis, i.e., all of the same order of magnitude for all three species. In natural sediments such a distinction will be difficult, if not impossible, to make, so that the distinction may be useful only in studies of the mechanism and kinetics

of uptake. Nevertheless failure to recognize this problem between the total sediment, the contaminated fraction, and the available fraction may be the cause of potentially large variations in calculated bioaccumulation factors. It is also important to note that bioaccumulation factors ignore the fact that it is less probable for an organism to consume a contaminated particle if it is present in small amounts. Consequently, the concentration independence of bioaccumulation factors has a lower limit, which should be investigated.

The measured bioaccumulation factor may be influenced by the feeding rate of the organisms, in part as affected by the behavior of the organisms in the sediment. Decreased activity of the organisms results in a decreased feeding rate, and may, therefore, lead to lower uptake. Observations of Tubifex and Capitella gave no reason to believe that these organisms behaved abnormally during the experiments. Some decreased activity of Nephtys may have occurred, however, but proof is lacking.

The results obtained fit in well with the little information that appears to be available concerning the bioaccumulation factors for DDT in soil- or sediment-ingesting organisms (Pimentel 1971, from which the following was cited). According to Stringer and Pickard (1964) earthworm (Lumbricus terrestris and other species) populations reflect DDT concentrations in soil: in soils containing 26.6 ppm, 4.1 ppm, and 3.6 ppm of DDT, the organisms averaged about 14 ppm, 7 ppm, and 3 ppm, respectively. Assuming that a steady state between the worms and their environment had been reached, bioaccumulation factors of 0.5 to 2 are indicated, varying little with the concentration in the environment. Hunt (1965) found earthworms to accumulate 141 ppm and 157 ppm of DDT in soils containing 9.9 ppm and 19 ppm, respectively in two areas sprayed with DDT for control of Dutch elm disease. Again under the assumption that a steady state between the worms and their environment had been reached, bioaccumulation factors of 8 to 14 are calculated. Earthworms in a cotton field exhibited a bioaccumulation factor for DDT of 11 (USDI, 1965). This same study showed a bioaccumulation factor in slugs of about 18. The amphipod Pontoporeia affinis concentrated about 0.014 ppm of DDT, DDE, and TDE in Lake Michigan sediments to about 0.41 ppm in the body, so that the bioaccumulation factor in this species is about 30 (Hickey et al., 1966).

Little is known about the kinetics of DDT uptake and loss by deposit feeders. An important factor concerning the applicability of the bioaccumulation concept is the time it takes for organisms to reach a steady state.

Fish appear to reach a steady state according to Hansen (1966; in Dustman and Stickel, 1969). Pinfish, for example, reached a steady-state body concentration of DDT of 12 ppm within two weeks when exposed to 0.001 ppm DDT in water. When the water concentration was 0.0001 ppm, the steady state was also reached within two weeks, at a body burden of 4 ppm. Thus the bioaccumulation factor varied from 12,000 to 40,000. (Bioaccumulation factors depend, of course, on the species of fish and are not necessarily all that high.) However, when fish are part of an ecosystem containing contaminated sediment, the DDT concentrations found in the fish may be relatively much lower. Croker and Wilson (1965) reported on a tidal marsh habitat treated with 0.2 lb/acre of DDT: surface water and ditch, 0.3 to 4.0 ppm; sediment, as high as 3.5 ppm (dry weight); vegetation, as high as 75 ppm (dry weight may be contributed to by deposition?); five species of fish, 4 to 58 ppm. In the Lake Michigan study mentioned earlier (Hickey et al., 1966), fish exhibited concentrations of 3.4 to 5.6 ppm, or about ten times the concentration in the amphipod.

In order to assess the application of the results for establishing dredged material disposal criteria, some consideration has been given here to the extent to which benthic deposit feeders can act as pesticide transfer agents in marine food webs. This pathway has apparently received little investigative attention in the past. Indeed the entire trophic concentration concept in aquatic food webs is being scrutinized in view of study results which suggest that the concept be modified to explain some observed pollutant distributions (Hamelink et al., 1971). In addition, evidence from National Science Foundation, International Decade of Ocean Exploration (NSF/IDOE), Pollutant Transfer Program suggests that trophic accumulation of chlorinated hydrocarbons is but one of the factors affecting the concentrations of these compounds in marine organisms (Duce et al., 1974). Other factors recognized in these studies were partition coefficients of individual compounds, variations in organisms lipid content, and characteristics of integument, cell surfaces, and excretion pathways. Consequently, the environmental impact of pesticide accumulation by benthic organisms feeding on pesticide contaminated dredged material cannot be completely assessed at this time. Data must be gathered concerning the importance of various

deposit-feeding species in marine food chains before the true magnitude of pesticide transport via the deposit feeder-predator pathway can be estimated. Furthermore, the degradation rate of pesticide in dredged material after it has been discharged, the degradation rate of pesticide in deposit-feeding organisms, the rate of pesticide recycling between organisms and sediment, and the rate of sediment movement from the disposal site must all be estimated to evaluate thoroughly whether sediment-adsorbed pesticides in dredged material pose problems in the marine environment. Thus it is evident that a much more extensive program of research is required than was envisaged by the present project. However, some positive statements can be made on the basis of the results and the scant information found in the literature.

Although it would be advisable, as a minimum, to measure the concentrations of DDT and its metabolites in naturally occurring contaminated sediments and their infauna, it is possible that bioaccumulation factors in deposit-feeding annelids will be of the same order of magnitude as those found in this study, probably about 10. These bioaccumulation factors are generally lower than those found when DDT is accumulated directly from the water column, for example, by fish, by algae (see e.g., Vance and Drummond, 1969) or by some Daphnia species (Priester, 1965). Based on these studies it would appear that any ecological significance of DDT in sediments is dwarfed by the demonstrated significance of DDT in the water column for those species investigated.

V. CONCLUSIONS

The basic objective of this study was to determine the availability of DDT and its degradation products DDD and DDE to selective and nonselective deposit feeders from interstitial water and from ingested detritus (for selective feeders) or from detritus plus clay particles (for nonselective feeders). A second objective was to determine whether uptake and accumulation, if occurring, are controlled by internal regulation, or if lack of a control mechanism results in continuing accumulation. The results show that the objectives were met under laboratory conditions. Specifically it is concluded:

1. When exposed to sediments containing DDT, Tubifex tubifex, Capitella capitata, and Nephtys californiensis accumulate the pesticide, indicating that at least a portion of sediment-adsorbed DDT is available to these deposit-feeding infauna.

2. Experiments with Tubifex and Capitella indicated that DDT was approximately equally available from organic and inorganic sediment components. Although Nephtys has been reported to be a selective feeder, this species was apparently able to accumulate a body burden of DDT from the inorganic sediment component.

3. Interstitial water may be an insignificant source of DDT, because DDT may be quantitatively adsorbed on clay and probably also on detritus.

4. Maximum levels of DDT accumulation were reached by Tubifex and by Capitella within 30 days, whereas a maximum level was attained by Nephtys at about 70-80 days. Thus a steady state indicating that some type of internal balance was attained between pesticide concentrations in the sediment and in the organisms, precluding long-term gradual increases of DDT and its metabolites in their body tissues.

5. Bioaccumulation factors calculated for Tubifex and for Nephtys were less than 10 and for Capitella about 50, when related to the total sediment. If only the tagged (contaminated) fraction of the sediment is considered as the source, all bioaccumulation factors were between 0.01 and 0.10. These values include any DDT which may have been in the gut, but still are of the same magnitude as those inferred for terrestrial annelids.

6. In contrast to DDT accumulation from water by aquatic organisms, DDT accumulation from sediments in the annelids used in this study proceeds at a slower rate, requires a longer time to reach a steady state, and results in lower maximum body burdens than would occur for the same concentration of DDT in water.

7. A great deal of additional research is required to allow more complete quantification of the environmental impact of the sediment-bottom feeder pathway for the introduction of DDT into the foodweb.

VI. RECOMMENDATIONS

The following recommendations are made with regard to additional studies to achieve the objectives of the DMRP.

1. Results obtained in laboratory studies utilizing artificial sediments do not necessarily yield valid extrapolations to natural systems. In particular, both qualitative and quantitative generalization of the bioaccumulation factor concept for the uptake of DDT as well as other persistent pesticides by benthic organisms are needed. Specific questions that should be addressed are:

a. Is a steady state reached in contaminated natural sediments as well as in artificial sediments?

b. If so, how much time will pass until the steady state is reached?

c. What is the relationship between the bioaccumulation factor and the DDT concentration in the sediment?

d. Is there a relationship between the bioaccumulation factor and the composition of the sediment, particularly with regard to particle size distribution and the relative amounts of organic and inorganic components?

The first two questions can be answered by means of uptake experiments in contaminated sediments from different areas and of different compositions, and with pesticide-free organisms. Sampling does not have to be extensive, but should be sufficient to indicate the steady state concentrations in the organisms. By carefully analyzing these same sediments it may also be possible to obtain an answer to the last question. The biggest problem would be the determination of the DDT concentrations in the organic and inorganic fractions separately. However, it may be possible to separate these fractions by means of a liquid of suitable density that will not desorb DDT. The third question may be answered by these experiments if a wide enough range of pesticide concentrations is present in the experimental sediments. If this is not the case, experiments with tagged natural sediments are indicated.

It is possible that some indication of the results of such experiments may be obtained by analyzing the infauna of the sediments for pesticides and comparing the results with the pesticide concentrations of the sediments. Sampling should be done several times over an eight-week period (for example, every two weeks), once during late spring and once during late autumn.

2. In order to better assess the significance of pesticide accumulation in benthic organisms, the biomagnification concept should be studied further. Such studies should include the determination of the significance of various benthic species in food webs, as well as accumulation by species characteristic of a variety of feeding types.

3. Detailed mechanistic studies should be initiated to enhance predictive capability. The details of such studies should be based upon conceptual models but with consideration of possible nonlinear mechanisms and more than one pool in the organisms.

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APPENDIX A
SEDIMENT TAGGING STUDIES

In order to determine the efficiency of tagging clay, sand, and cereal with DDT, the following procedure was used:

a. Weigh out four 10 g portions of clay (sand) (2 g portions of seven day aged cereal) and transfer to 250 ml stoppered flasks.

b. Measure 100 ml deionized water for each run.

c. Prepare a moderately concentrated solution of ^{14}C-labeled DDT in acetone.

d. Measure out enough DDT-in-acetone to contain:

   d1. 0.01 μg DDT (1 ppb relative to clay)
   d2. 0.03 μg DDT (3 ppb " " ")
   d3. 0.1 μg DDT (10 ppb " " ")
   d4. 0.3 μg DDT (30 ppb " " ")
   and mix with the water aliquots.

e. Immediately add the water to the clay in the flasks and shake for approximately two hours.

f. Transfer the contents of the flasks to 40 ml centrifuge tubes and centrifuge.

g. Remove the supernate, determine its volume (weight), and analyze for DDT.

h. Remove about one half of the precipitate and analyze for DDT.

i. Transfer remainder of precipitates to fresh flasks, add 50 ml water, and shake for approximately two hours.

j. Separate water and solids.

k. Analyze solids for DDT (keep the water).

The results are shown in Table A1. It is seen that DDT is quantitatively adsorbed from the spiking solution on the clay, and almost quantitatively on the cereal, whereas adsorption on sand is rather poor. Apparently a very small amount of DDT may be desorbed from clay but the separation of clay and water may not have been entirely quantitative. It is noted, from the back

TABLE A1

RESULTS OF DDT-TAGGING STUDIES WITH SAND, CLAY, AND CEREAL

DDT concentration relative to clay (ppb)	Percent of DDT left in water	Percent of DDT desorbed on water
CLAY		
1	0	2
3	0	2
10	0	3
30	0	2
SAND		
1	23	34
3	22	28
10	25	49
30	40	22
CEREAL		
1	0	-
3	3	-
10	1	-
30	3	-

extraction experiments with sand, that true equilibrium may not have been present. Since the procedure for tagging the sediments to be used in the uptake experiments does not involve the violent back extraction, it was concluded that no measurable DDT would be present in the interstitial water, the small amount of DDT-in-water in equilibrium with cereal being adsorbed on clay during sediment preparation.

An attempt was made to resolve the question if there might be any movement of DDT between clay and cereal. Since it was deemed impossible to make this determination under the exact conditions of the uptake experiments, the following experiment was conducted. A simple apparatus was constructed as shown in Figure A1. A measured amount of one sediment component, tagged, is placed in the top portion of the apparatus on a glass wool support plug. Similarly the second component (untagged) is placed in the bottom portion. The T near the bottom of the upper section of the column provides an option of sampling the water that percolates through the sediment in the lower portion. Also elution of top and bottom sediments may be done separately. Sediments used were a 10% clay-sand mixture and cereal. Elutions were done with 50 ml of water. After tagging of the upper sediment with enough DDT suspension to wet the sediment completely, elution was done with two successive 50 ml portions of water. The results showed that with tagged cereal the first eluate contained no measurable DDT. However, there was no release of DDT from clay. The observation that only the first eluate from cereal contained a small amount of DDT is interpreted as resulting from channeling or the removal of pockets of water containing unadsorbed DDT. The conclusion was reached that transport of DDT through water between clay and cereal is either very slow or does not occur. Potential transport by bacterial action was not considered in this experiment, and complete resolution of the question was not achieved.

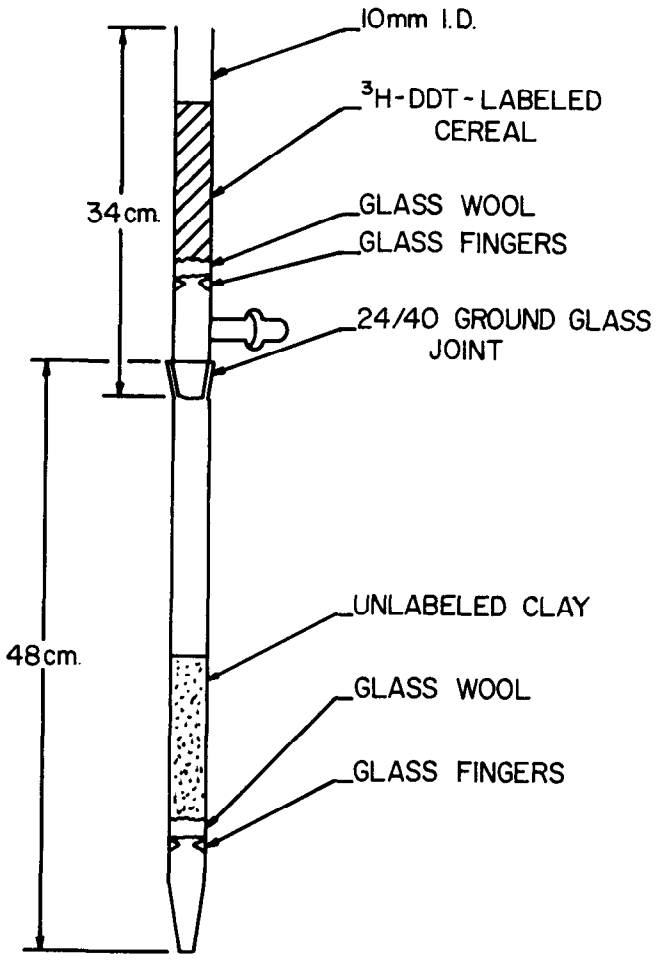

Figure A1 Apparatus to study potential transfer of pesticides between sediment components

APPENDIX B

COMPILATION OF DATA

TABLE B1

UPTAKE OF LABELED DDT (AND METABOLITES) BY TUBIFEX TUBIFEX FROM A TAGGED ARTIFICIAL SEDIMENT (FIRST EXPERIMENT)[1]

Exposure Time[2] (days)	Tank 2				Tank 3			
	Sample Wt. (g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	Total DDT (ng/g)	Sample Wt. (g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	Total DDT (ng/g)
0.5	0.474	0.09	0.17	0.26	0.244	0.23	0.12	0.35
1.0	0.770	0.77	0.16	0.93	0.306	0.11	0.03	0.14
2.1	0.287	1.19	0.28	1.47	0.330	0.53	0.18	0.71
3.0	0.523	1.30	0.42	1.72	0.296	1.31	0.17	1.48
5.1	0.373	2.46	0.91	3.37	0.268	0.42	1.01	1.43
8.2	0.333	1.03	1.02	2.05	0.278	0.84	1.33	2.17
12.1	0.592	0.77	1.18	1.95	0.403	2.2	2.15	4.35
17.9	0.377	0.63	1.87	2.50	0.211	0.79	1.28	2.07
22.8	0.246	1.0	6.74	7.74	0.369	0.54	3.37	3.91

(1) Sediment composition: 98% silica sand
1% clay with 100 ng ^{3}H-DDT per gram
1% aged cereal with 100 ng ^{14}C-DDT per gram.

(2) Experiment was started at 10:00 a.m., November 30, 1974.

Table B1 (Concluded)

Exposure Time (days)	Avg ^{14}C-DDT Concentration (ng/g)	Avg ^{3}H-DDT Concentration (ng/g)	Avg DDT Concentration (ng/g)	$\frac{^{14}C \text{ Concentration}}{^{3}H \text{ Concentration}}$
0.5	0.16	0.14	0.30	1.14
1.0	0.44	0.10	0.54	4.40
2.1	0.86	0.23	1.09	3.74
3.0	1.30	0.30	1.60	4.33
5.1	1.44	0.96	2.40	1.50
8.2	0.94	0.93	1.87	1.01
12.1	1.48	1.66	3.14	0.89
17.9	0.71	1.58	2.29	0.45
22.8	0.77	5.06	5.83	0.15

TABLE B2

UPTAKE OF LABELED DDT (AND METABOLITES) BY TUBIFEX TUBIFEX FROM A TAGGED ARTIFICIAL SEDIMENT (SECOND EXPERIMENT)[1]

Exposure Time[2] (days)	Tank 2				Tank 3			
	Sample Wt. (g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	Total DDT (ng/g)	Sample Wt. (g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	Total DDT (ng/g)
0.5	0.657	0.05	0.70	0.75	0.573	0.09	0.61	0.70
1.0	0.526	0.17	0.89	1.06	0.524	0.08	0.46	0.54
2.1	0.467	0.34	16.0[3]		0.431	0.23	0.72	0.95
3.0	0.375	0.37	3.44	3.81	0.243	0.66	4.16	4.82
5.2	0.416	0.55	1.77	1.33	0.919	0.86	3.00	3.86
9.0	0.365	0.79	2.35	3.14	0.226	0.80	2.44	3.24
12.0	0.406	3.23	2.76	6.04	0.469	1.92	1.26	3.28
17.0	0.410	1.22	1.63	2.85	0.567	0.67	1.03	1.70
22.0	0.709	1.51	2.58	4.09	0.375	1.12	1.52	1.94
27.0	0.542	1.18	2.37	3.01	0.560	1.34	2.02	2.77
32.0	0.637	1.49	1.68	3.17	0.260	0.99	1.66	2.65
37.0	0.445	1.86	2.16	4.02	0.419	1.64	1.58	3.22
46.0	0.408	2.16	3.16	5.32	0.531	1.83	3.00	4.83
52.0	0.544	1.78	2.47	4.25	1.454	1.79	2.30	4.29

(1) Sediment composition: 98% silica sand
1% clay with 100 ng ^{14}C-DDT per gram
1% aged cereal with 100 ng ^{3}H-DDT per gram.

(2) Experiment was started at 10:00 a.m., January 14, 1975.

(3) Value to be discarded as lying outside 3 σ limit (3.8 σ).

Table B2 (Concluded)

Exposure Time (days)	Avg ^{14}C-DDT Concentration (ng/g)	Avg ^{3}H-DDT Concentration (ng/g)	Avg DDT Concentration (ng/g)	$\frac{^{14}C \text{ Concentration}}{^{3}H \text{ Concentration}}$
0.5	0.07	0.66	0.73	9.43
1.0	0.12	0.68	0.80	5.67
2.1	0.28	0.72	1.00	2.57
3.0	0.52	3.80	4.32	7.31
5.2	0.70	2.38	3.08	3.40
9.0	0.80	2.40	3.20	3.00
12.0	2.52	1.51	4.03	0.60
17.0	0.94	1.33	2.27	1.41
22.0	1.32	2.05	3.37	1.55
27.0	1.26	2.20	3.46	1.75
32.0	1.24	1.67	2.91	1.35
37.0	1.75	1.87	3.62	1.07
46.0	2.00	3.08	5.08	1.54
52.0	1.78	2.38	4.16	1.34

TABLE B3

DDT CONTENT OF SEDIMENT DURING SECOND UPTAKE EXPERIMENT WITH TUBIFEX TUBIFEX

Exposure Time (days)	Tank 2		Tank 3		Avg	
	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)
17.0	0.45	0.40	0.34	0.34	0.39	0.37
32.0	0.46	0.41	0.50	0.45	0.48	0.43

TABLE B4

UPTAKE OF LABELED DDT (AND METABOLITES) BY CAPITELLA CAPITATA FROM TAGGED ARTIFICIAL SEDIMENT(1)

Exposure Time(2) (days)	Tank 2				Tank 3			
	Sample Wt. (g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	Total DDT (ng/g)	Sample Wt. (g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	Total DDT (ng/g)
0.5	0.394	0.76	1.00	1.76	0.590	0.61	0.85	1.46
1.0	0.772	0.94	1.93	2.87	0.279	0.63	1.34	1.97
2.0	0.500	1.49	2.02	3.51	0.305	1.52	2.66	4.18
4.0	0.401	1.47	1.76	3.23	0.322	1.90	4.79	6.69
8.0	0.290	20.0	11.9	31.9	0.173	4.61	4.31	8.92
12.0	0.215	11.5	11.9	23.4	0.163	19.7	14.7	34.4
18.2	0.204	25.5	16.6	42.1	0.575	14.4	8.30	22.7
22.0	0.295	26.8	18.9	45.7	0.159	29.0	20.9	49.9
27.2	0.249	37.1	23.2	60.3	0.283	25.1	51.0	76.1
33.0	0.263	48.2	31.8	80.0	0.203	80.6	56.9	137.5
37.0	0.345	48.1	25.5	73.6	0.280	64.7	42.1	106.8
42.0	0.261	27.0	25.9	52.9	0.263	51.1	42.9	94.0
49.0	0.235	66.7	54.0	120.7	0.202	53.7	39.0	92.7
54.9	0.148	99.3	46.3	145.6	0.254	67.0	42.2	109.2

(1) Sediment composition: 99.8% beach sand (baked at 400° C)
0.1% aged cereal with 1000 ng ^{14}C-DDT per gram
0.1% clay with 1000 ng ^{3}H-DDT per gram.

(2) Experiment was started at 10:00 a.m., April 4, 1975.

Table B4 (Concluded)

Exposure Time (days)	Avg ^{14}C-DDT Concentration (ng/g)	Avg ^{3}H-DDT Concentration (ng/g)	Avg DDT Concentration (ng/g)	$\frac{^{14}C \text{ Concentration}}{^{3}H \text{ Concentration}}$
0.5	0.68	0.92	1.60	0.74
1.0	0.78	1.63	2.41	0.48
2.0	1.50	2.36	3.86	0.64
4.0	1.68	3.27	4.95	0.51
8.0	12.3	8.1	20.4	1.52
12.0	15.6	13.3	28.9	1.17
18.2	20.0	12.4	32.4	1.61
22.0	27.9	20.4	48.3	1.37
27.2	31.1	37.1	68.2	0.84
33.0	64.4	44.3	108.7	1.45
37.0	56.4	33.8	90.2	1.67
42.0	39.0	34.4	73.4	1.13
49.0	60.2	46.5	106.7	1.29
54.9	83.1	44.2	127.3	1.88

TABLE B5

DDT CONTENT OF SEDIMENT DURING UPTAKE EXPERIMENT WITH CAPITELLA CAPITATA

Exposure Time (days)	Tank 9		Tank 11		Avg	
	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)	^{14}C-DDT (ng/g)	^{3}H-DDT (ng/g)
0.5	0.48	0.26	0.47	0.27	0.47	0.27
4.0	0.45	0.36	0.14	0.15	0.29	0.25
18.2	0.36	0.47	0.37	0.40	0.37	0.43
18.2	0.45	0.35	0.39	0.29	0.42	0.32
33.0	0.25	0.27	0.31	0.34	0.28	0.31
54.9	0.32	0.33	0.24	0.24	0.28	0.29
54.9	0.27	0.19				

TABLE B6

UPTAKE OF LABELED DDT BY NEPHTYS CALIFORNIENSIS FROM TAGGED NATURAL SEDIMENT

Exposure Time (days)	Tank 2		Tank 3		Avg DDT Concentration (ng/g)
	Sample Wt. (g)	DDT Concentration (ng/g)	Sample Wt. (g)	DDT Concentration (ng/g)	
0.5	1.25	0.16	1.46	0.18	0.17
1.0	1.74	0.28	0.57	0.18	0.23
2.0	1.28	0.28	1.01	0.17	0.22
4.0	1.13	0.43	1.16	0.63	0.53
8.0	2.05	0.40	1.56	0.88	0.64
12.0	0.58	0.43	0.27	1.42	0.92
16.0	1.38	0.92	1.09	3.14	2.03
24.4	1.34	1.89	0.26	1.86	1.87
33.0	0.83	2.37	1.50	1.08	1.72
41.1	1.45	1.47	1.15	4.66	3.1
50.3	1.36	1.3	1.13	5.1	3.2
60.1	1.54	2.8	0.74	2.2	2.5
70.0	0.82	2.3	0.93	6.9	4.6
80.0	0.65	6.4	0.89	3.9	5.2
105	0.29	5.6	1.08	6.1	5.9
121	0.67	6.6	1.41	3.1	4.9
127	1.88	3.9			3.9

TABLE B7

UPTAKE OF LABELED DDT (AND METABOLITES) BY NEPHTYS CALIFORNIENSIS FROM NATURAL SEDIMENT MIXED WITH TAGGED CLAY

Exposure Time (days)	Tank 1		Tank 2		Avg DDT Concentration (ng/g)
	Sample Wt. (g)	DDT Concentration (ng/g)	Sample Wt. (g)	DDT Concentration (ng/g)	
2.0	0.73	1.0	*		1.0
4.1	2.95	1.5	3.89	1.1	1.3
9.0	1.65	2.0	*		2.0
12.9	1.79	2.5	2.19	2.8	2.7
18.0	0.91	1.9	2.70	1.1	1.5
23.0	1.11	3.8	1.81	2.9	3.3

*Specimens lost.

TABLE B8

DDT CONTENT OF SEDIMENT AND WATER DURING UPTAKE EXPERIMENT WITH NEPHTYS CALIFORNIENSIS

	Exposure Time (days)	DDT Concentration Tank 2 (ng/g)	Tank 3 (ng/g)	Avg (ng/g)	Control Tank
Sediment	4.0	0.30		(0.30)	0
	50.3	0.21	0.42	0.31	0
	80.0	0.20	0.25	0.23	
	80.0*	0.18	0.22	0.20	
	105	0.23	0.25	0.24	
	121	0.27	0.16	0.21	
Sediment (short run)	2.0	0.31			
	23.0	0.40			
Water**	50.3	0	0		
	70.0	0	0		0
	90.0	0			0

*Duplicate analysis.
**Concentrations in ng/l.

TABLE B9

DDT AND METABOLITE COMPOSITION OF TISSUE AND SEDIMENT SAMPLES DURING UPTAKE EXPERIMENTS WITH DIFFERENT SPECIES OF ANNELIDS

(FRACTION OF TOTAL)

	Exposure Time (days)	DDT		DDD		DDE	
		^{14}C clay	^{3}H cer	^{14}C clay	^{3}H cer	^{14}C clay	^{3}H cer
A. Tubifex tubifex							
Tank 2 Sediment	17	0.922	0.904	0.074	0.084	0.004	0.001
	22	0.883	0.884	0.116	0.108	0.001	0.008
	32	0.671	0.654	0.327	0.340	0.003	0.006
	37	0.626	0.627	0.369	0.373	0.005	0
Tank 3 Sediment	17	0.920	0.903	0.078	0.077	0.001	0.020
	22	0.891	0.878	0.109	0.116	0	0.006
	32	0.803	0.710	0.195	0.279	0.002	0.010
	37	0.690	0.729	0.304	0.271	0.006	0
Average Sediment	17	0.921	0.903	0.076	0.080	0.002	0.010
	22	0.887	0.881	0.112	0.112	0	0.007
	32	0.737	0.682	0.261	0.310	0.002	0.008
	37	0.658	0.678	0.336	0.322	0.006	0
Tank 2 Tissue	32	0.040	0.288	0.960	0.321	0	0.391
	37	0.019	0	0.904	1.00	0.077	0
	46	0	0.348	1.00	0.258	0	0.393
	52	0.034	0.055	0.966	0.777	0	0.168
Tank 3 Tissue	32	0.608	0.414	0.392	0.302	0	0.284
	37	0	0	1.00	1.00	0	0
	46	0.007	0.207	0.993	0.568	0	0.225
	52	0.582	0.496	0.418	0.418	0	0.087
Average Tissue	32	0.324	0.351	0.676	0.312	0	0.338
	37	0.010	0	0.952	1.00	0.038	0
	46	0.004	0.278	0.278	0.413	0	0.309
	52	0.308	0.276	0.692	0.598	0	0.128
B. Capitella capitata							
Tank 9 Sediment	0.5	0.973	0.931	0.022	0.043	0.004	0.026
	4	0.937	0.942	0.056	0.045	0.006	0.013
	18	0.449	0.491	0.545	0.494	0.006	0.015
	33	0.226	0.203	0.755	0.697	0.019	0.100
	55	0.117	0.183	0.845	0.752	0.038	0.065
Tank 11 Sediment	0.5	0.929	0.924	0.050	0.048	0.021	0.028
	4	0.927	0.929	0.063	0.054	0.010	0.017
	18	0.514	0.624	0.477	0.361	0.009	0.014
	33	0.259	0.353	0.715	0.601	0.026	0.046
	55	0.222	0.322	0.732	0.636	0.046	0.041

Table B9 (Concluded)

	Exposure Time (days)	DDT ^{14}C clay	DDT ^{3}H cer	DDD ^{14}C clay	DDD ^{3}H cer	DDE ^{14}C clay	DDE ^{3}H cer
Average Sediment	0.5	0.951	0.928	0.036	0.046	0.013	0.027
	4	0.932	0.936	0.060	0.050	0.008	0.014
	18	0.482	0.558	0.511	0.428	0.008	0.015
	33	0.242	0.278	0.735	0.649	0.023	0.073
	55	0.170	0.252	0.788	0.694	0.042	0.053
Tank 9 Tissue	4	0.642	0.413	0.358	0.413	0	0.173
	18	0.517	0.532	0.483	0.468	0	0
	55	0.057	0.070	0.924	0.898	0.019	0.032
Tank 11 Tissue	4	0.756	0.825	0.226	0.147	0.017	0.028
	18	0.421	0.400	0.579	0.571	0	0.029
	55	0.227	0.257	0.760	0.722	0.013	0.021
Average Tissue	4	0.699	0.619	0.292	0.280	0.009	0.101
	18	0.469	0.466	0.531	0.520	0	0.015
	55	0.142	0.164	0.842	0.810	0.016	0.026

	Exposure Time (days)	Tank 2 DDT	Tank 2 DDD	Tank 2 DDE	Tank 3 DDT	Tank 3 DDD	Tank 3 DDE
C. Nephtys californiensis							
Sediment	4	0.987	0.010	0.002	0.979	0.011	0.009
	50	0.877	0.114	0.009	0.709	0.288	0.003
	121	0.778	0.203	0.019	0.550	0.419	0.031
Tissue	4				0.980	0.020	0
	50	0.918	0.082	0	0.830	0.166	0.005
	70	0.878	0.098	0.024	0.924	0.068	0.008
	80	0.522	0.466	0.012	0.090	0.908	0.002
	105	0.793	0.181	0.026	0.738	0.238	0.025
	121	0.559	9.433	0.007			
		Average Sediment			Average Tissue		
	4	0.983	0.010	0.006	0.980	0.020	0
	50	0.793	0.201	0.006	0.874	0.124	0.003
	70				0.901	0.080	0.016
	80				0.306	0.687	0.007
	105				0.766	0.210	0.026
	121	0.664	0.311	0.025	0.559	0.433	0.007

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Nathans, M W
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